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The complete mitochondrial genome of *Coralliophila richardi* (P. Fischer, 1882) (Neogastropoda: Muricidae: Coralliophilinae)

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ABSTRACT

The complete mitochondrial genome of *Coralliophila richardi* was determined using Illumina sequencing and found to be a double-stranded circular molecule 15,553 bp in length with a GC content of 34.9%. It contains 13 protein-coding genes, 2 ribosomal RNA genes and 21 tRNA genes with the same strand orientation as in other neogastropods. The tRNA gene for arginine was not identified in the mitogenome, although this amino acid is present in each of the proteins coded by the mitogenome. The mitochondrial gene order in *C. richardi* differs from that of all other neogastropods in having three translocations of tRNA genes, and two translocations of a tRNA gene and an adjacent protein coding gene. This is the first report of the translocation of protein-coding genes within the mitogenome of any neogastropod. Phylogenetic analyses show Coralliophilinae emerging either as a weakly supported sister taxon to the remaining subfamilies of Muricidae for which mitogenome data was available (ML), a result compatible with its former rank as family Coralliophilidae, or unresolved (BI) from the two clades containing these muricid subfamilies due to long branch bias generated by accelerated mutation rates within the coralliophiline mitogenome. In a separate analysis based solely on partial 12SrRNA, the bathyal *C. richardi* emerged as a well-supported sister taxon to *C. brevis*, a shallow water species with a partially overlapping geographic range.

Additional Keywords: Coralliophagy, deep sea, phylogenetics, mitochondrial rearrangements, long branch attraction

INTRODUCTION

The neogastropod subfamily Coralliophilinae comprises a lineage of highly specialized ecto- and endoparasites of anthozoan cnidarians, including sea anemones, gorgonians, as well as solitary and reef building corals. Some live externally on corals (e.g., *Coralliophila* H. and A. Adams, 1853, *Quoyula* Iredale, 1912), while others (such as *Magilus* Montfort, 1810 and *Leptoconchus* Rüppell, 1835) become embedded in hard and soft corals (e.g., Taylor, 1976; Brawley and Adey 1982; Guzmán, 1988; Massin, 2000). This subfamily has a worldwide distribution, primarily in tropical and temperate seas, and ranges from subtidal to upper bathyal depths (~1,500 m). It contains approximately 250–275 species, assigned to 11 Recent genera (MolluscaBase eds. 2022). Coralliophilinae had a broad geographic range during the Eocene, including Australia (Darragh, 2017), Kamchatka, Russia (Oleinik, 2001) and the southeastern United States (Dockery, 1980). Earlier records have been reported from the Campanian (Sohl, 1964) and Maastrichtian (Late Cretaceous) of the eastern and southern United States (Sohl and Koch, 1984; Sohl, 1960) [as *Latiaxis*] and from Upper Albian (Early Cretaceous) deposits of Madagascar (Besarie and Collignon, 1972) [as *Rapa*].

The close relationship of this lineage to Muricidae has long been recognized. Muricidae are characterized by their shell morphology and diagnostic anatomical features, among them a distinctive rachiglossan radula, paired accessory salivary glands, purple hypobranchial gland secretion, the presence of an anal gland and an accessory boring organ in the sole of the foot. However, the clade containing *Coralliophila* and related genera lacks a radula and radular apparatus, accessory salivary glands, accessory boring organ, and differs in other features of the alimentary system due to a suctorial mode of feeding on cnidarians. This clade had been accorded the rank of family Coralliophilidae Chenu, 1859 [= Magilidae Thiele, 1925; = Rapidae Kuroda, 1941] (e.g., Cossmann, 1903; Wenz, 1941; Radwin and D'Attilio, 1971; Kantor, 1995; Ponder, 1974), but more recent studies have included Coralliophilinae as one of the subfamilies within Muricidae (e.g., Ponder and Warén, 1988; Bouchet *et al.*, 2017; MolluscaBase eds., 2022).

While the monophyly of Coralliophilinae is strongly supported by anatomical characters and in several molecular studies, efforts to discern phylogenetic relationships of this clade to the remaining subfamilies of Muricidae have produced inconsistent results that vary depending on the taxa included and the characters or genes used. Several

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molecular studies with limited taxon representation have indicated a close relationship between Coralliophilinae and Rapaninae (Harasewych *et al.*, 1997: fig. 6A, B; Oliverio and Mariottini, 2001: fig. 14; Oliverio *et al.*, 2002: fig. 3), but this relationship was not recovered in Oliverio *et al.* (2009: fig. 1). The broadest study of muricid phylogeny to date (Barco *et al.*, 2012) (Figure 1), partitioned the family into two major clades, one containing Rapaninae, Coralliophilinae, and its sister subfamily Ergalataxinae, the other clade including representatives of 8 of the 9 remaining recognized subfamilies of Muricidae (lacking only Tripterotyphinae) (MolluscaBase eds. 2022).

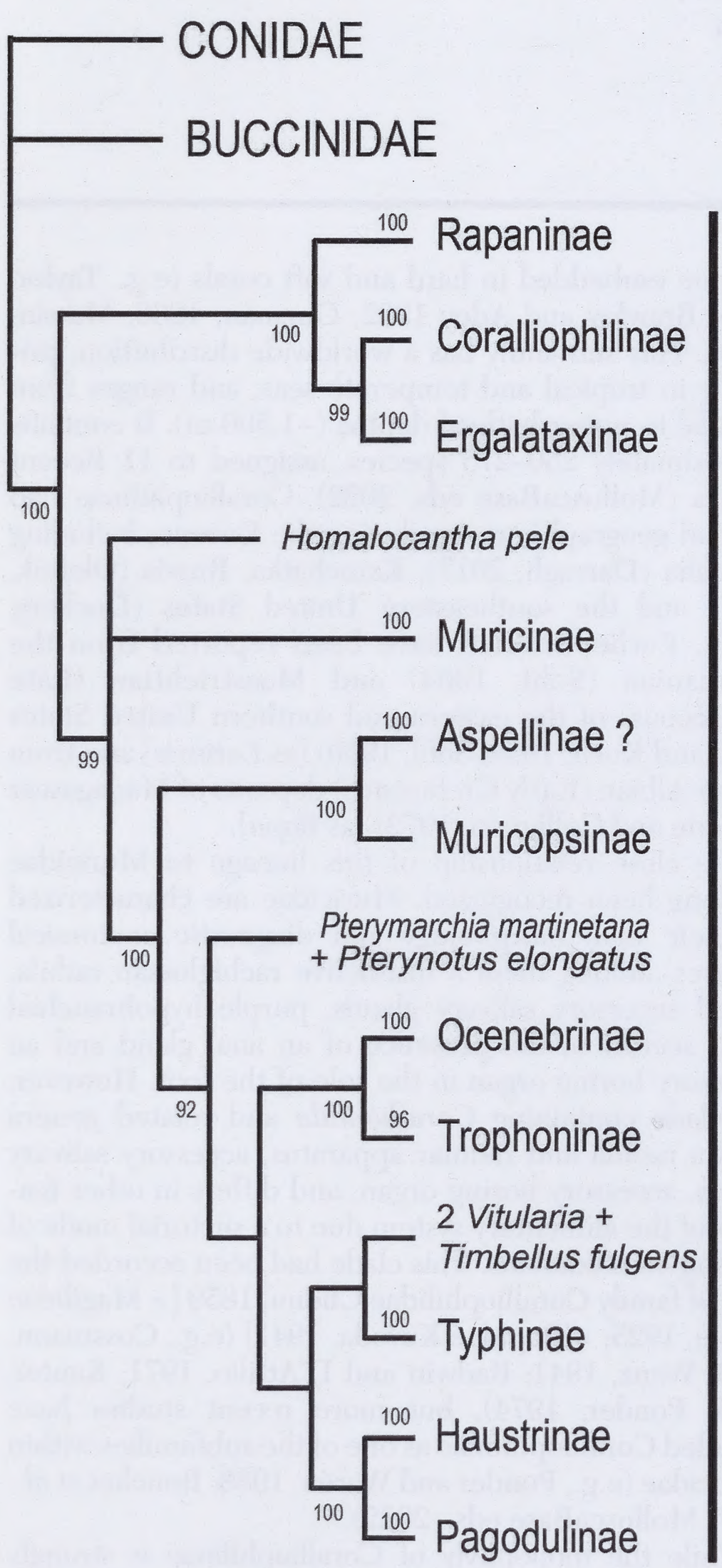

Figure 1. Phylogenetic relationships among the subfamilies of Muricidae summarized from the Bayesian analysis of Barco *et al.* (2012: fig. 1, lacking only Tripterotyphinae). Species level taxa in this tree are those that emerged outside of the subfamilies to which they are currently assigned (MolluscaBase eds. 2022).

In this study, we report the complete mitochondrial genome of *Coralliophila richardi* (P. Fischer, 1882), a wide-ranging species inhabiting upper bathyal depths from the Gulf of Mexico to the Mediterranean Sea. It is the first complete mitogenome of a species of Coralliophilinae to be determined. Various features of this mitogenome are compared to those of other muricids, and the phylogenetic position of Coralliophilinae within Muricidae is analyzed based on amino acid sequences of the protein-coding genes and nucleotide sequences of the ribosomal RNA genes. We also infer the placement of this species within Coralliophilinae by adding a portion of its12S rRNA gene sequence to a dataset from 42 species of coralliophilines analyzed by Oliverio *et al.* (2009).

MATERIALS AND METHODS

DNA Extraction and Sanger Sequencing: A section of foot muscle (~ 40 mg) was dissected from an ethanol-preserved specimen of *Coralliophila richardi* [USNM 1526878] collected at the Many Mounds site, west of Sanibel, Florida (26°12′27″N, 84°43′34″W) in 478 m by the ROV Odysseus, aboard NOAA Ship Nancy Foster, Cruise NG1708, Dive 10, on August 19, 2017. Genomic DNA was extracted using the Qiagen DNeasy kit (Qiagen, Valencia, CA, USA) using the manufacturer's spin-column protocol for animal tissues, with the initial lysis step extended to 24 hours at 56°C with continuous agitation. Portions of the cytochrome *c* oxidase subunit I (COX1) and 16S rRNA genes were PCR-amplified and Sanger-sequenced using the primers and protocols in Harasewych (2019).

Library Construction and Illumina Sequencing: Raw genomic DNA was quantified using a Qubit 4 Fluorometer (Invitrogen, Waltham, MA) before library preparation. NEBNext® Ultra™ II FS DNA Library Prep Kit for Illumina® (New England Biolabs, Ipswich, MA) was used for library construction with the following modifications to the standard protocol for inputs ≤ 100 ng: reaction volume was reduced by half; 10 minute incubation for fragmentation/end prep; undiluted Y-Yoke adaptor (Glenn *et al.*, 2019) was used instead of NEBNext Adaptor for Illumina; 30 minute incubation for adaptor ligation; bead cleanup with KAPA Pure Beads (Roche, Branchburg, NJ); iTru i5 and i7 indices (Glenn *et al.*, 2019) instead of NEBNext Oligos for Illumina; and 10 PCR cycles. Library quality and quantity were checked with Qubit 4 Fluorometer and 4200 Tapestation (Agilent, Santa Clara, CA) before standardizing and multiplexing. Next Generation Sequencing was performed on one lane of a S4 flowcell on a NovaSeq 6000 (Illumina, San Diego, CA) with 150 bp PE reads (Admera Health Biopharma Services, South Plainfield, NJ).

Assembly: Low quality reads were removed using TrimGalore v. 0.6.3 (https://github.com/FelixKrueger/TrimGalore), with thresholds for minimum Phred scores set to 20 and minimum read lengths set to 20 bp. The *Coralliophila richardi* mitogenome was assembled with Geneious ® 11.1.4 (http://www.geneious.com) using the following protocol: Sanger-sequenced COX1 and 16S rRNA gene fragments were each used as scaffolds using the "map to reference" tool with "minimum overlap identity" set to 98–99% and "minimum overlap" set to 30–40 bp. Five to eight rounds of iterations at medium sensitivity were needed before an assembled contig of >15kb was obtained for each scaffold. The two contigs were then aligned and found to be identical. The aligned sequence was then circularized.

Genome Annotation: Mitochondrial elements were annotated using MITOS2 (Donath *et al.*, 2019), tRNAscan-SE 2.0 (Chan *et al.*, 2021) and the ORF finder in Geneious ® 11.1.4. Amino acid sequences of protein-coding genes were compared to those of corresponding genes in five gastropod species (identified by † in Table 1) in order to select between alternative start and stop positions indicated by MITOS2 and Geneious ORF finder.

Phylogenetic Analyses: Phylogenetic analyses were conducted using data from the complete mitogenome of *Coralliophila richardi* as well as from an additional 22 species, with two Littorinimorpha outgroups as well as 20 neogastropods, including representatives of all seven living neogastropod superfamilies, and five of twelve currently recognized subfamilies within Muricidae for which mitogenome data are available in GenBank (Table 1).

Phylogenetic trees were constructed using amino acid sequences of the 13 protein-coding genes and nucleotide sequences of both ribosomal genes. The Bayesian inference (BI) analyses were run in PhyloBayes (Lartillot *et al.*, 2013) under the CAT-GTR model, discarding constant sites ("-dc" option). Two independent MCMC chains were run, sampling every cycle, until convergence (maxdiff < 0.1, meandiff < 0.1, and effective sample size > 100). All runs consisted of >10,000 cycles and consensus trees were obtained after discarding the first 10% of cycles as burn-in. For the maximum likelihood (ML) analyses, best-fit partition schemes, with each partition and its corresponding empirical substitution matrices, were determined using options "-m TESTONLYMERGE" and the Bayesian information criterion (BIC) in ModelFinder (Kalyaanamoorthy *et al.*, 2017). The resulting matrix and scheme with models were run using the IQ-TREE (Nguyen, *et al.*, 2015) platform under a combination of rapid hill-climbing and stochastic perturbation methods. A total of one thousand pseudo-replicates of ultrafast bootstrap (UFBoot) were performed to assess the robustness of the inferred trees.

In order to determine the relationship of *Coralliophila richardi* within Coralliophilinae, its 12S rRNA gene was aligned against partial 12S rRNA sequences of 34 species of Coralliophilinae that were used to produce the phylogenetic tree in Oliverio *et al.* (2009: table 1, fig. 1). Sequences of the 12S rRNA gene of taxa selected to serve as outgroups (*Buccinum undatum*, *Drupella rugosa*, and *Rapana venosa*) were obtained from their complete mitogenomes (Table 1). The ML analyses of the resulting 12S matrix were run using IQ-TREE as described above but with "TESTNEW" model selection strategy, while the BI analyses were run using MrBayes 3.2 (Ronquist *et al.*, 2012) with the GTR-I-G model and default parameters, four simultaneous Monte Carlo Markov chains (MCMC) for 10 million generations were run sampling every 1,000 generations, and discarding the first 25% of generations as burn-in.

RESULTS

Mitogenome content and organization: After filtering and removing low-quality data, 16,029,980 reads (Phred scores ≥ 20, length ≥ 20 bp) remained with an average length of 139.8 bp (SD 24.1). Of these, 3,199 mapped to the mitochondrial genome. Coverage ranged from 5X to 156X per site (mean = 28.2; SD = 19.3).

The mitochondrial genome of *Coralliophila richardi* (GenBank Acc. no. OM307660) is a double-stranded circular molecule 15,553 bp in length and is within the range reported for other Muricidae (Table 1). It is composed of 27.0% A, 38.1% T, 15.3% C, and 19.6% G. Thirteen protein-coding genes, 2 ribosomal RNA genes, and 21 tRNA genes were identified within this mitogenome (Table 2, Figure 2). The tRNA gene for arginine was not identified, although this amino acid is present in each of the proteins coded by the mitogenome. Twenty-eight of the genes are coded on the heavy (+) strand and eight tRNA genes are coded on the light (-) strand, including the clusters CGW (tRNA-C, tRNA-G, tRNA-W), MYQE (tRNA-M, tRNA-Y, tRNA-Q, tRNA-E) and tRNA-T.

Protein coding genes constitute 72.0% of the mitogenome (11,192 bp). The most common start codon is ATG, which occurs in 9 genes, with ATA occurring in two genes (NAD1 and NAD4), GTG in NAD2 and ATT in NAD3. The TAG stop codon occurs in six genes (COX1, COX2, ATP6, MAD1, NAD4L, NAD5), TAA stop codon in three genes (NAD2, ATP8, COX3), with the genes for NAD6, CytB, NAD4 and NAD3 ending with the truncated stop codon T that is completed by post transcriptional polyadenylation to T(AA) (Donath *et al.*, 2019). The ribosomal RNA genes comprise 14.0% of the mitogenome (2,178 bp), while 21 tRNA's have a length of 1,353 bp (8.7% of the mitogenome). Gene overlaps total 145 bp at 14 gene junctures, with the largest (45 bp) shared by the genes for NAD4 and tRNA-H. Eleven intergenic regions occupy 6.3% of the mitogenome, totaling 973 bp (1–592 bp), with the largest between the genes for COX3 and tRNA-K.

The mitochondrial gene order in *C. richardi* differs from that shared by most neogastropods, including all muricids reported to date (Cunha *et al.*, 2009: 210, fig. 1). Rearrangements of the gene order of *C. richardi*

Table 1. List of taxa used in phylogenetic analyses, their GenBank accession numbers, source of sequence data, and size of the mitogenome. Taxon names as well as their superfamily, family, and subfamily assignments have been updated according to WoRMS (2020). † indicates species used to compare amino acid sequences of protein-coding genes during annotation.

Superfamily	Family	Subfamily	Taxon	GenBank No.	Source	length (bp)
OUTRGROUPS - LITTORINIMORPHA						
Stromboidea	Strombidae		*Aliger gigas* †	NC_024932	Marques *et al.*, 2016	15,461
Tonnoidea	Charoniidae		*Charonia lampas*	NC_037188	Cho *et al.*, 2017	15,330
NEOGASTROPODA						
Volutoidea	Cancellariidae		*Bivetiella cancellata*	NC_013241	Cunha *et al.*, 2009	16,648
Volutoidea	Volutidae	Calliotectinae	*Neptuneopsis gilchristi*	MN125492	Harasewych *et al.*, 2019	15,312
Volutoidea	Volutidae	Amoriinae	*Melo melo*	MN462590	Zhong *et al.*, 2019c	15,721
Turbinelloidea	Costellariidae		*Costapex baldwinae*	MW044625	Uribe *et al.*, 2021	15,321
Olivoidea	Ancellariidae		*Amalda northlandica*	NC_014403	McComish *et al.*, 2010	15,354
Mitroidea	Mitridae	Isarinae	*Isara chinensis*	MT415926	Qiao *et al.*, 2020	16,238
Unassigned			*Babylonia lutosa*	NC_028628	Xiong *et al.*, 2015	15,346
Buccinoidea	Columbellidae		*Columbella adansoni*	KP716637	Osca *et al.*, 2015	16,272
Buccinoidea	Melongenidae		*Hemifusus tuba*	MN462591	Zhong *et al.*, 2019b	15,483
Buccinoidea	Buccinidae	Buccininae	*Buccinum undatum* †	NC_040940	Jonsson *et al.*, 2019	15,265
Connoidea	Conidae		*Californiconus californicus*	NC_032377	Uribe *et al.*, 2016	15,444
Connoidea	Conidae		*Conus ventricosus*	KX63251	Uribe *et al.*, 2016	15,534
Muricoidea	Muricidae	Coralliophilinae	*Coralliophila richardi*	OM307660	This study	15,553
Muricoidea	Muricidae	Rapaninae	*Rapana venosa* †	KM213962	Sun & Yang, 2016	15,271
Muricoidea	Muricidae	Rapaninae	*Menathais tuberosa*	NC_031405	Sung *et al.*, 2016	15,294
Muricoidea	Muricidae	Ergalataxinae	*Drupella rugosa* †	OK052648	Ma, unpublished	16,343
Muricoidea	Muricidae	Muricinae	*Murex trapa* †	MN462589	Zhong *et al.*, 2019a	15,408
Muricoidea	Muricidae	Muricinae	*Chicoreus asianus*	MN793976	Zhong *et al.*, 2020	15,361
Muricoidea	Muricidae	Pagodulinae	*Boreotrophon candelabrum*	NC_046505	Tian *et al.*, 2019	15,265
Muricoidea	Muricidae	Ocenebrinae	*Ocinebrellus inornatus*	NC_046577	Hao *et al.*, 2019a	15,324
Muricoidea	Muricidae	Ocenebrinae	*Ceratostoma burnetti*	NC_046569	Hao *et al.*, 2019b	15,334

Table 2. Position (start, stop), strand direction (+/-) and lengths (bp) of the genes in the mitochondrial genome of *Coralliophila richardi* (P. Fischer, 1882) [15,553 bp; GenBank OM307660], initiation and termination codons (Init/Term) for protein-coding genes, as well as their amino acid sequence lengths (Laa). Standard abbreviations for protein coding genes are used. Both three and one letter abbreviations are listed for tRNA's, along with the codon used. The numbers of intergenic nucleotides (Ign) are shown. Negative numbers indicate overlap of genes. †- indicates that start or stop differs from that predicted by MITOS2. ‡ - indicates that tRNA same in MITOS2 and tRNAscan-SE.

Gene	Start	Stop	Strand Directionn	Length (bp)	Init /Term	Laa	Ign
COX1 †	1	1,542	+	1,540	ATG/TAG	513	–2
tRNA-Asn (N) (gtt) ‡	1,541	1,607	+	67			0
tRNA-Ser (S1) (gct)	1,608	1,671	+	64			0
NAD2 †	1,672	2,709	+	1,038	GTG/TAA	345	1
COX2	2,711	3,400	+	690	ATG/TAG	229	–7
ATP6	3,394	4,083	+	690	ATG/TAG	229	23
tRNA-Cys (C) (gca) ‡	4,167	4,107	–	61			–4
tRNA-Gly (G) (tcc)	4,231	4,164	–	68			0
tRNA-Trp (W) (tca) ‡	4,295	4,232	–	64			62
tRNA-Asp (D) (gtc) ‡	4,358	4,421	+	64			0
ATP8 †	4,422	4,595	+	174	ATG/TAA	57	5
tRNA-Met (M) (cat)	4,665	4,601	–	65			–3
tRNA-Tyr (Y) (gta)	4,724	4,663	–	62			–3
tRNA-Gln (Q) (caa)	4,784	4,722	–	63			–2
tRNA-Glu (E) (ttc) ‡	4,846	4,783	–	64			62
12S rDNA	4,909	5,752	+	844			–6
tRNA-Val (V) (tac) ‡	5,747	5,808	+	62			–25
16S rDNA	5,784	7,117	+	1,334			–25
tRNA-Leu (L1) (tag) ‡	7,093	7,159	+	67			0
tRNA-Leu (L2) (taa) ‡	7,160	7,221	+	62			–3
NAD1	7,219	8,163	+	945	ATA/TAG	314	0
tRNA-Pro (P) (tgg) ‡	8,164	8,226	+	63			0
NAD6 †	8,227	8,722	+	496	ATG/T(AA)	165	0
CYT B †	8,723	9,863	+	1,141	ATG/T(AA)	380	0
tRNA-Ser (S2) (tga)	9,864	9,928	+	65			–2
tRNA-Thr (T) (tgt) ‡	9,991	9,927	–	65			1
NAD4L	9,993	10,289	+	297	ATG/TAG	98	68
NAD4	10,358	11,693	+	1,336	ATA/T(AA)	445	–45
tRNA-His (H) (gtg) ‡	11,649	11,713	+	65			0
NAD5	11,714	13,429	+	1,716	ATG/TAG	571	–11
tRNA-Phe (F) (gaa) ‡	13,419	13,483	+	65			104
COX3	13,588	14,367	+	780	ATG/TAA	259	592
tRNA-Lys (K) (ttt)	14,960	15,028	+	69			–7
tRNA-Ala (A) (tgc) ‡	15,022	15,085	+	64			52
tRNA-Arg (R)(tcg)	gene not detected in mitogenome						
tRNA-Ile (I) (gat) ‡	15,138	15,201	+	64			3
NAD3 †	15,205	15,553	+	349	ATT/T(AA)		0

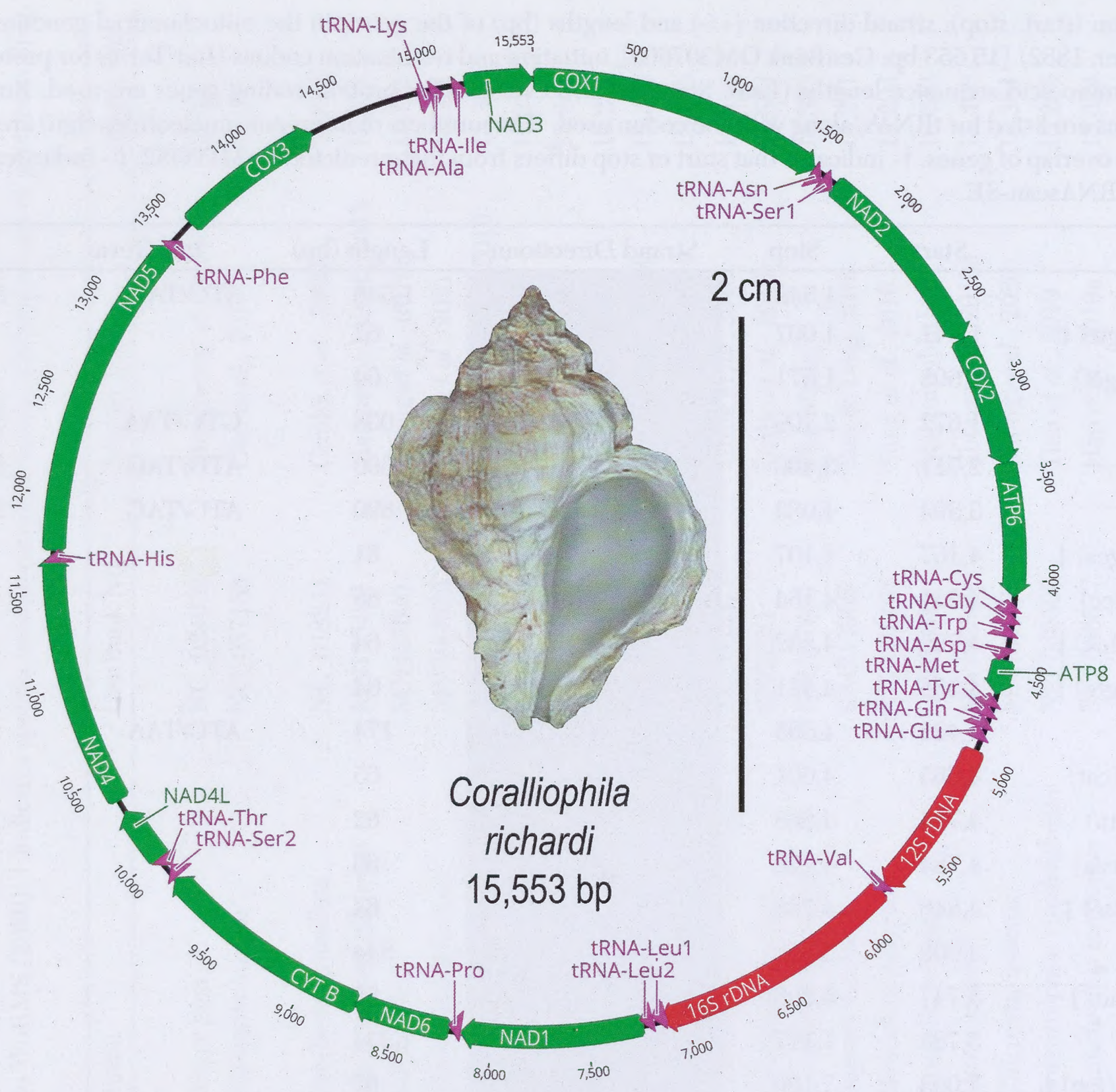

Figure 2. Map of the mitochondrial genome of *Coralliophila richardi* (GenBank Acc. no. OM307660). Arrows indicate the direction of transcription. Protein-coding genes are in green, ribosomal genes in red and transfer RNA genes in purple.

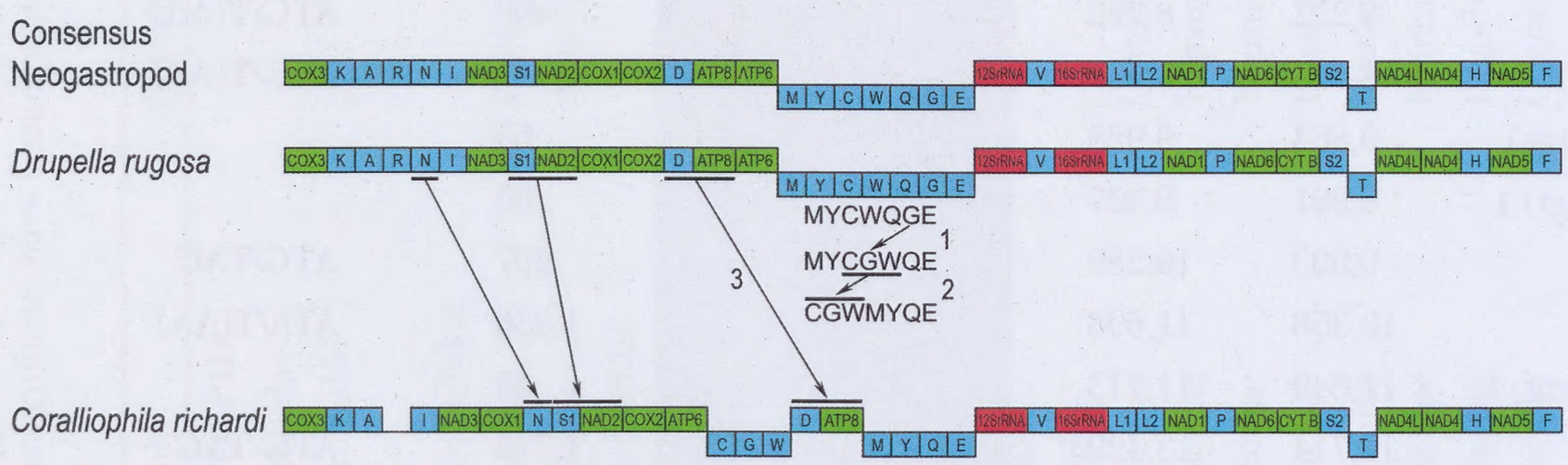
Consensus
Neogastropod
COX3 K A R N I NAD3 S1 NAD2 COX1 COX2 D ATP8 ATP6
M Y C W Q G E
12SrRNA V 16SrRNA L1 L2 NAD1 P NAD6 CYT B S2
T
NAD4L NAD4 H NAD5 F
Drupella rugosa
COX3 K A R N I NAD3 S1 NAD2 COX1 COX2 D ATP8 ATP6
M Y C W Q G E
12SrRNA V 16SrRNA L1 L2 NAD1 P NAD6 CYT B S2
T
NAD4L NAD4 H NAD5 F
MYCWQGE
MYCGWQE
1
CGWMYQE
2
3
Coralliophila richardi
COX3 K A I NAD3 COX1 N S1 NAD2 COX2 ATP6
D ATP8
12SrRNA V 16SrRNA L1 L2 NAD1 P NAD6 CYT B S2
NAD4L NAD4 H NAD5 F
C G W
M Y Q E
T

Figure 3. Linear representations of gene order in all Muricidae studied to date, exemplified by *Drupella rugosa* as well as in *Coralliophila richardi*, indicating rearrangements in gene order. The gene order of the hypothetical ancestral neogastropod is shown for comparison.

include the translocation of the tRNA-G gene within the MYCWQGE tRNA cluster (Figure 3, 1) followed by the translocation of the newly adjacent tRNA genes CGW also within the same cluster (Figure 3, 2) followed by the translocation of the genes for tRNA-D and ATP8 into the same cluster of tRNA's (Figure 3, 3). In addition, there are two independent translocations, one of the gene for tRNA-N, the other for the genes for tRNA-S1 and NAD2 to positions between COX1 and COX2.

Phylogenetic Analyses: Phylogenetic analyses of the concatenated amino acid sequences for all mitochondrial protein-coding genes and nucleotide sequences of both ribosomal genes using maximum likelihood

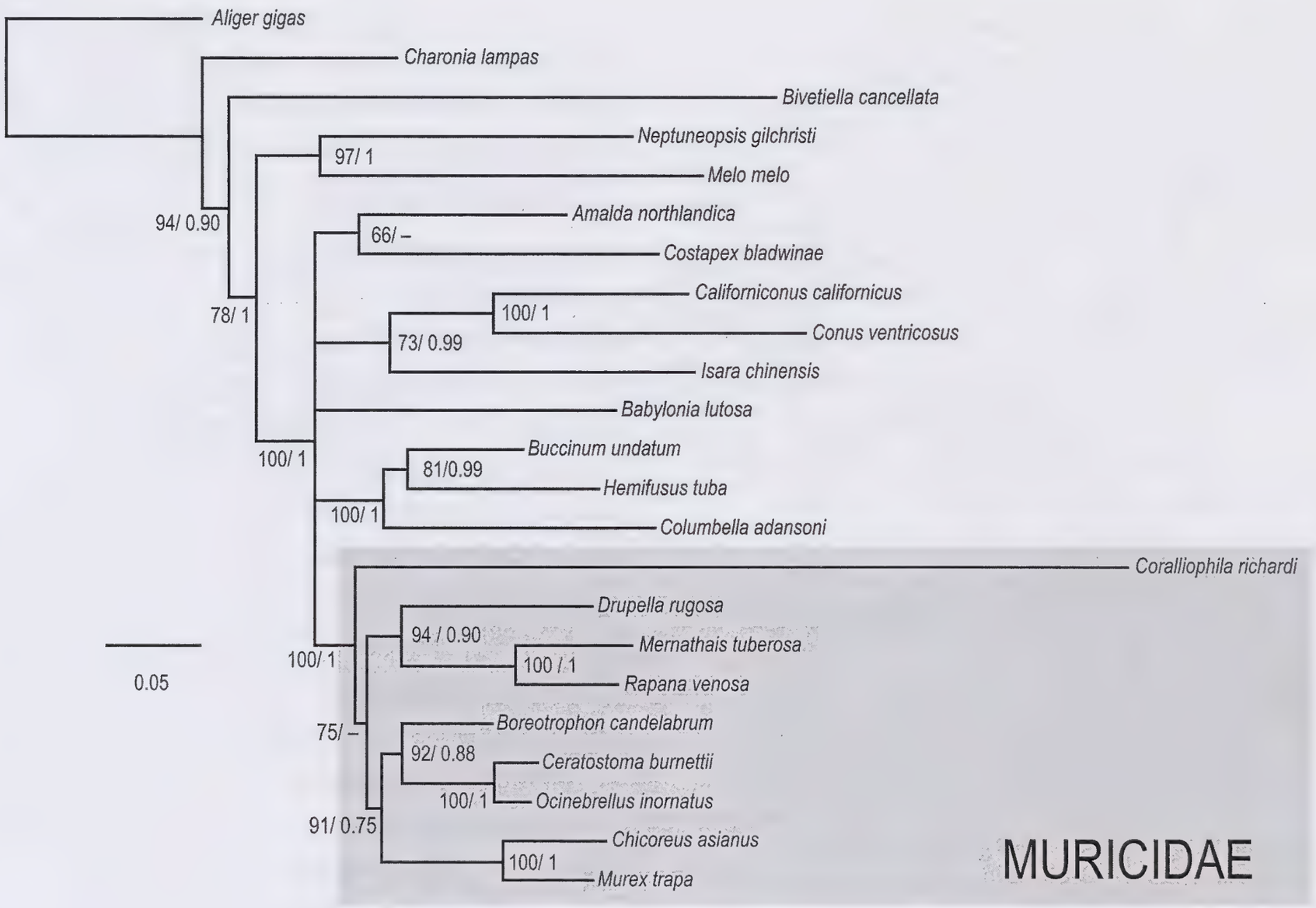

Figure 4. Phylogenetic relationships of Coralliophilinae based on maximum likelihood and Bayesian analyses of amino acid sequences of mitochondrial protein-coding genes and nucleotide sequences of both ribosomal genes. Branch support shown as maximum likelihood bootstrap values (when ≥ 50)/Bayesian posterior probability (when ≥ 0.7).

analysis produced a single tree with weak support for relationships among neogastropod superfamilies other than Volutoidea (Figure 4). The tree based on Bayesian inference was largely congruent with the ML tree, with relationships among the neogastropod superfamilies other than Volutoidea also either poorly supported or unresolved. Coralliophilinae emerged either as a weakly supported sister taxon to the remaining subfamilies of Muricidae for which mitogenome data was available (ML), or unresolved (BI) from the two clades of muricid subfamilies indicated in the tree of Barco *et al.* (2010).

Both maximum likelihood and Bayesian inference analyses of partial 12S rRNA sequences of 34 coralliophiline taxa from Oliverio *et al.* (2009) to which the *Coralliophila richardi* sequence was added reveal a well-supported sister group relationship between *C. richardi* and *C. brevis* (Figure 5).

DISCUSSION

The mitochondrial genome of *Coralliophila richardi*, the first representative of the subfamily Coralliophilinae for which such data are available, is comparable in size to those of other neogastropods but has an increased number (14 vs. 6) of overlapping regions between genes that are larger in size (2–45 bp vs.1–29 bp), as well as having a different gene order. Most neogastropods share a consensus gene order (Figure 3). Known exceptions include several instances of gene rearrangement within the superfamily Conoidea involving the translocation of either a single tRNA gene or rarely of a cluster of two or three adjacent tRNA genes, with a single report of both a translocation and inversion of a single tRNA gene (Cunha *et al.*, 2009: fig. 1; Uribe *et al.*, 2016: fig. 1; 2018: fig. 2). Rearrangements within the mitogenome of *C. richardi* are the first record of a translocation of protein-coding genes within the mitogenome of any neogastropod. The inability to identify the transfer RNA gene for the amino acid arginine (tRNA-R) within this mitogenome may be a consequence of the close proximity of tRNA-R gene to the tRNA-N gene (separated by only 3 intergenic nucleotides in *Rapana* and *Drupella*), which has undergone translocation to a position between COX1 and COX2.

Phylogenetic analyses of data from available mitogenome sequences corroborate the relationships among muricid subfamilies reported by Barco *et al.* (2012) (Figure 1), with exception of the placement of Coralliophilinae. Analyses of phylogenetic relationships using both maximum likelihood and Bayesian inference (Figure 4) strongly support the monophyly of Coralliophilinae and the remaining muricid subfamilies. Maximum likelihood analyses indicate that Coralliophilinae is the sister taxon to the remaining muricid subfamilies, a result compatible with its former rank as family Coralliophilidae, while Bayesian inference indicates an unresolved trichotomy between Coralliophilinae, a clade including Ergalataxinae and Rapaninae, and a clade including representatives of Muricinae, Pagodulinae and Ocenebrinae. Coralliophilinae is not included in the clade containing Ergalataxinae and Rapaninae as

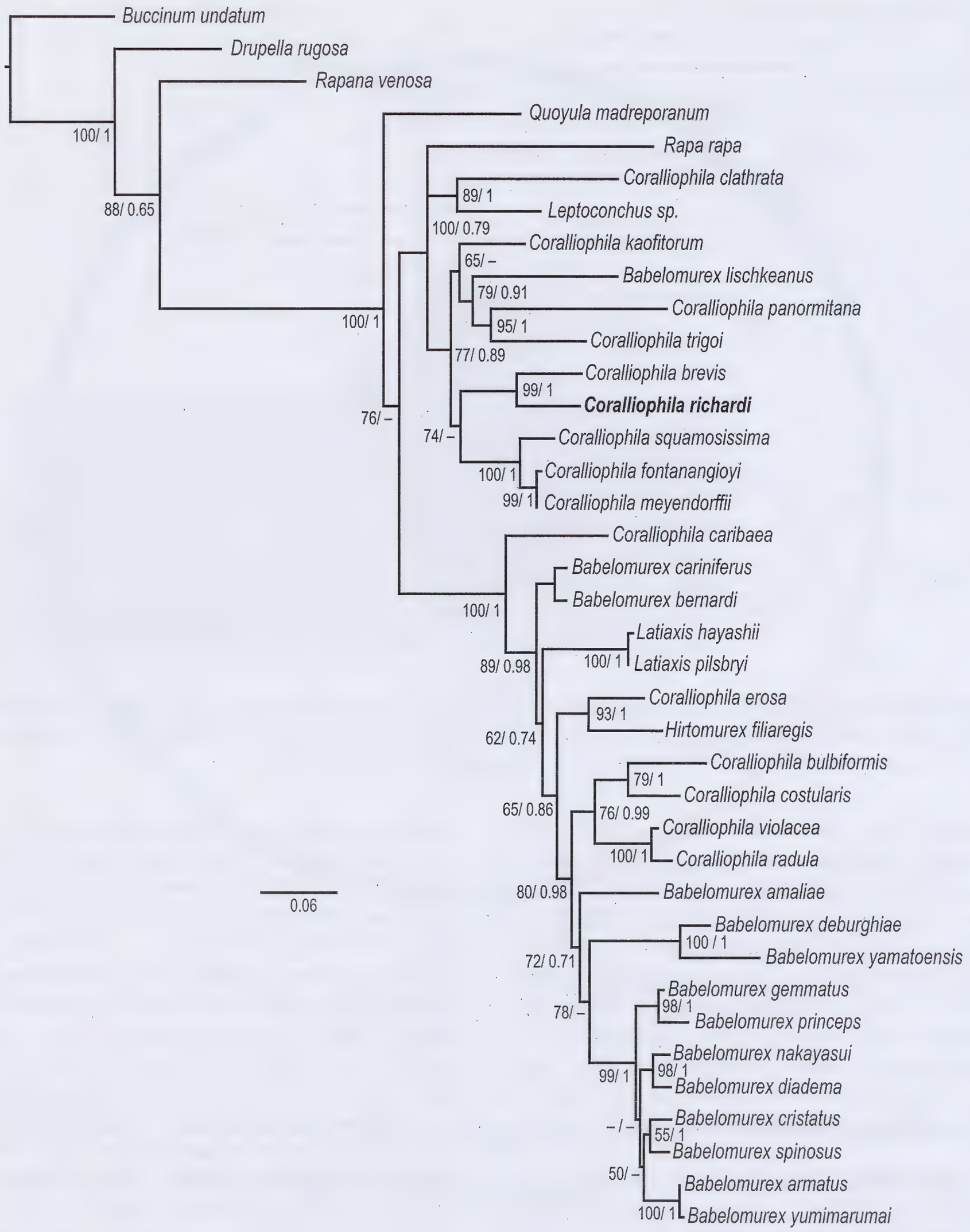

Figure 5. Phylogenetic relationships of *Coralliophila richardi* to other members of the Coralliophilinae based on Maximum Likelihood and Bayesian analyses of partial 12S rRNA sequences. Maximum Likelihood tree with branch support shown as maximum likelihood bootstrap value (when ≥ 50)/Bayesian posterior probability (when ≥ 0.70).

previously reported (Barco *et al.*, 2010; 2012). As noted by Barco *et al.* (2010:1037), Coralliophilinae are characterized by long branches in trees based on mitochondrial genes. This is evident in the trees reported here, which are based entirely on complete mitochondrial genomes.

Xu *et al.* (2006) reported a correlation between rearrangements in mitochondrial genomes and increased rates of sequence evolution, which may result in long branch artifacts when reconstructing phylogenies. Rearrangements in the mitochondrial gene order of *C. richardi* compared to other neogastropods likely contribute long branch artifacts that diminish resolution of its phylogenetic relationships based on mitochondrial genomes. Uribe *et al.* (2022) noted that phylogenetic analyses based on mitogenome sequences would likely be most robust when analyzing samples with identical or similar gene orders.

Like many sedentary species, Coralliophilinae have a protandric-hermaphroditic life history (Soong and Chen, 1991; Oliverio *et al.*, 2009). Davison (2006) reported that mutations in nuclear genes may accumulate at twice the rate in hermaphroditic as in gonochoristic mollusks, with a potentially higher rate associated with mitochondrial

genes, noting that protandrous species may evolve more rapidly than protogynous species. If, as suggested by Barco *et al.* (2010), this applies to coralliophilines, the use of nuclear genes to resolve phylogenetic relationships of Coralliophilinae may be less prone to long branch issues, but not entirely immune.

The taxonomic affinities of *Coralliophila richardi* have been questioned by Taviani *et al.* (2009), who noted that its shell morphology is unusual within Coralliophilinae and compared it to *Emozamia licina* (Hedley and Petterd, 1906) a deep-water western Pacific species. Our analyses of phylogenetic relationships among coralliophiline species based on partial sequences of the 12S rRNA gene published by Oliverio *et al.* (2009: table 1) to which the corresponding *C. richardi* sequence was added (Figure 5) reveal a well-supported sister group relationship between *C. richardi* and *Coralliophila brevis* (Blainville, 1832) a shallow-water species that ranges from the eastern Atlantic (Cantabrian Sea to Canary Islands) throughout the Mediterranean Sea. These two species overlap in the eastern portion of their geographical ranges, although *C. brevis* occurs in shallow water (to about 30 m), while *C. richardi* inabits bathyal depths (120–1,400 m).

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A unique new species of middle Eocene vermetiform gastropod from the Crockett Formation, Texas, USA

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ABSTRACT

A sample of well-preserved fossil vermetiform gastropods (worm snails) encrusting small solitary corals provides data on the larval shell, growth changes associated with fixation on substrate, and ornamentation of teleoconch of a small vermetiform species. This species differs from other vermetiforms in having a slender, high-spired larval shell covered with close-spaced uniform collabral ribbing, small diameter teleoconch tube, and substrate preference for attachment to living coral tissue. Settlement of larvae on living, small, solitary corals is shown by extensive overgrowth of coral skeleton onto the gastropod shell as it grew over the coral wall. The teleoconch tube is covered with narrow, smooth-surfaced annular bands and lacks longitudinal ribs or cancellate sculpturing. The protoconch of this species is unlike described protoconchs of species in family Vermetidae, suggesting that an origin for the species independent of the Vermetidae. A new genus and species, *Brazoverma brazina*, are erected for this fossil species.

Additional Keywords: worm snails, protoconch, fossil

INTRODUCTION

Specimens of a well-preserved fossil vermetiform gastropod (worm snail) with characters unlike those of known species in family Vermetidae are present in a stratigraphic deposit of Eocene age located in east-central Texas (Figure 1; 30.641099°, –96.520595°). The species occurs in richly fossiliferous mudstones of the late middle Eocene age Crockett Formation. The species is distinctive in having very well preserved protoconch/larval shell and occurs only encrusting the surfaces of small, free-living solitary corals that inhabited a mud-substrate ocean seafloor. The species has a life habit of larval shell attaching to a hard surface and producing a teleoconch tube of irregular form during subsequent growth, similar to the life habit of many vermetid gastropods. The sample contains many newly attached larval shells followed by short teleoconch shell growth onto the surface of a coral, as well as several specimens that grew out to maturity with long teleoconch tubes, providing a record of the ontogeny of this distinctive species.

Study of this sample was started with the goal of obtaining data useful for understanding the ancestry of fossil vermetid gastropods based on study of the larval shell, a character considered to be conservative and reliable for use in determining relationships for vermetid gastropods (Golding et al., 2014), and for many gastropod groups. However, the results revealed the presence of a previously unknown vermetiform gastropod with shell characters distinctive enough to suggest a different origin. Ontogeny of the new species is revealed by examination of 35 specimens (on 13 coral specimens) at growth stages ranging from initial attachment to maturity. This fossil vermetiform snail species has a millimeter-long larval shell consisting of a slender shell with tight helical coiling extending for several whorls. This is unlike the illustrated larval shells of living vermetid species (Keen, 1961; Gardner, 1947; Savazzi, 1996; Golding et al., 2014) and both the larval shell and teleoconch shell are dissimilar to the vermetiform turritellid *Vermicularia* (Bieler and Hadfield, 1990) and to species of vermetiform siliquariids (Bieler, 2004; Bieler and Simone, 2005). The topic of relationship is discussed and evaluated later in this report.

Study of vermetiform fossils is generally confusing and unstable because most of them consist of only segments of the irregular teleoconch tube produced by the mature gastropod (Keen, 1961, Gardner, 1947; Gould, 1994; Bieler and Petit, 2011). These segments do not provide a full characterization of a species, so there is a perception of great variability in shells and generalizations that the teleoconch can be encrusting or erect, have variable surface ornamentation, and vary in the development of internal laminae. Without data on their early ontogeny and identifying stable species characters, including larval shell and the growth changes associated with fixation, distinguishing named fossil species from each other and determining relationships among them is difficult. Despite these difficulties, the group has been the subject of study by many workers that are fascinated with these unusual gastropods. For living species of vermetids the degree of relationship can be aided with the use genetic data (Golding et al., 2014) but genetic data is not available for fossil species. Resolving the problem of identifying

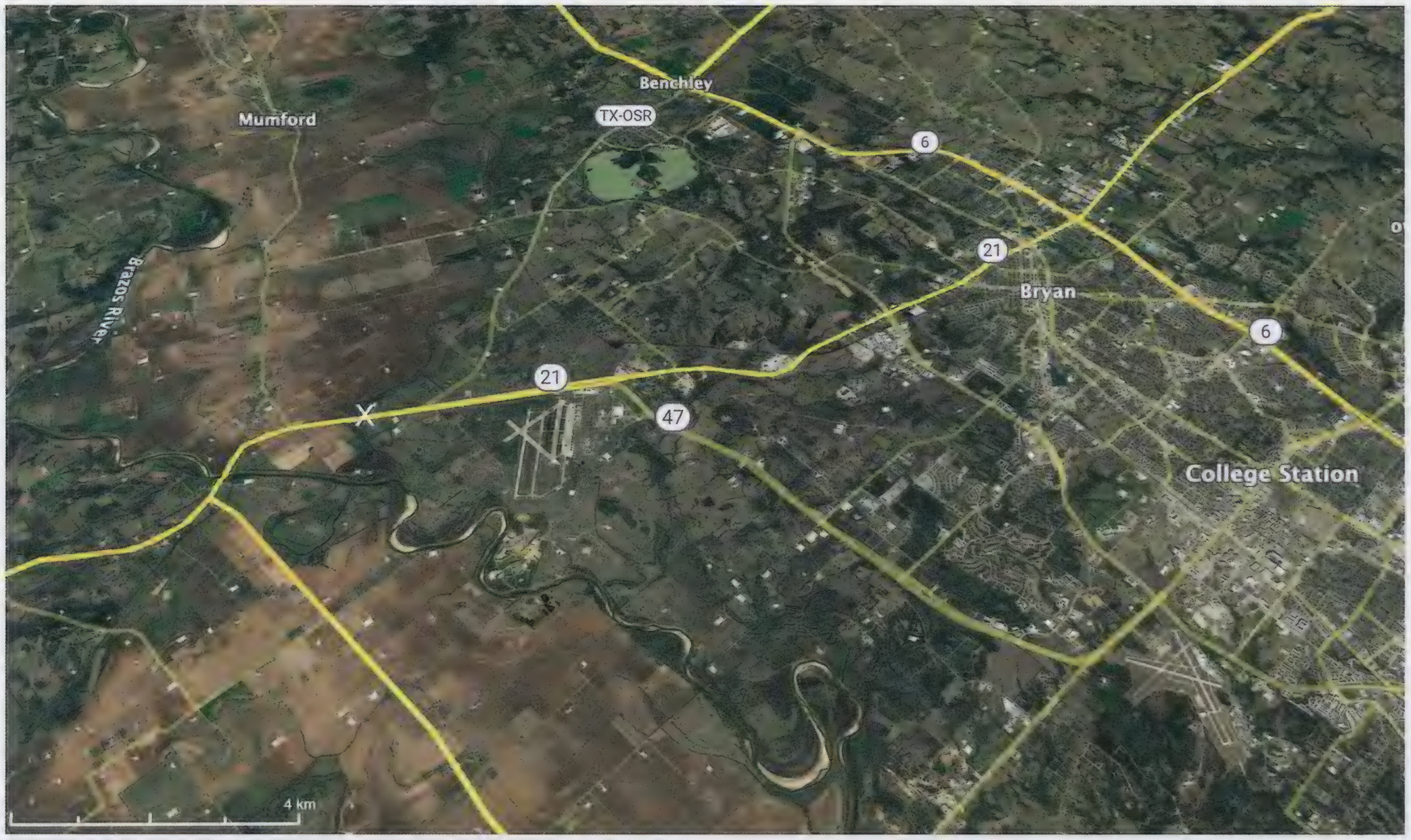


Figure 1. Location (marked by X) of sample at Hwy. 21 crossing of Little Brazos River, west of Bryan-College Station, Texas. Lat-Long: 30.641099, –96.520595. Map from Google Earth.

fossil species can be accomplished by determining the limits of variation and identification of stable characters useful for species identification.

MATERIALS AND METHODS

The sample providing fossils for this study comes from sediment exposed during the drilling of large diameter bores used for construction of pilings to support a highway bridge over the Little Brazos River in Brazos County, Texas. The fossil bed is a 2 m thick mudstone, collected at a depth of 5 m below ground surface on the banks of the river at the bridge site. It is a dark mudstone of the Wheelock Member of the Crockett Formation that overlies a unit with thick siderite-cemented beds having great geographic extent. This interval of the formation is exposed in an outcrop belt that extends 120 km northeast to Houston County, Texas and is correlated by macrofossil biostratigraphy (Stenzel, 1940) and by radiometric dating of volcanic ash beds (Heintz et al., 2015) associated with the mudstone bed and siderite-cemented beds. The thicker of two volcanic ash beds that underlie the very fossiliferous mudstone at the Little Brazos site is dated by $^{40}Ar/^{39}Ar$ method at 41.841 million years before present (Heintz et al., 2015).

Fossil-bearing sediments collected from the site were not exposed to weathering and contain scattered masses of pyrite formed during early diagenesis of the sediment. The sample preserves shells of both calcite and aragonite in original condition and shows no evidence of leaching. The bed has a massive character without lamination, indicative of bioturbation during the time of sediment accumulation. Fossils are dispersed in the sediment, with only a few, thin layers of shell concentrate and little or no indication of shell breakage by water transport. Biogenic shell fragmentation is common along with indications of predation. Both microfossils and macrofossils are abundant in the sediment, including organic-walled microfossils as well as mineral skeletal material. Some fossil fragmentation is the result of sediment compaction of this clay- and silt-rich sediment.

Laboratory processing of the sample was done by drying the sediment thoroughly, then immersing the sediment in water to produce disaggregation, then screening the loose material on a screen with 300 µm mesh openings. A second round of drying, wetting, and screening produced very clean specimens suitable for identification. This process could be repeated for increased cleanliness if desired. No additional processing was needed. Species sorting with the aid of a binocular microscope is best for the small species. Specimens were cleaned and illustrated with light photography using a Zeiss Stemi stereozoom microscope with phototube camera attachment and use of a desktop SEM Phenom XL imaging unit at 15 kV in low vacuum (60Pa) for examining shell microstructure.

Personal observation on modern species of *Vermicularia* and *Tenagodus* are based on samples collected from offshore reefs and banks in the northern Gulf of Mexico collected by the Department of Oceanography, Texas A&M University, and housed in the Marine Invertebrate Collection of the Texas A&M University Biodiversity Research and Teaching Collection, housed in College Station, Texas.

Repository Collection: Specimens used in this study, 35 gastropods encrusting 13 coral specimens, are deposited in the collections of the Non-vertebrate Paleontology Laboratory (NPL) of the Jackson School of Earth History, University of Texas, Austin, Texas.

SYSTEMATICS

Genus *Brazoverma* new genus
(Figures 2–11)

Type Species: *Brazoverma brazina* new species

Description: Characters of the type species, with shell consisting of small, slender, high-spired, tightly coiled larval shell of 4–5 whorls with closely spaced, fine collabral ribbing, and having a shiny shell surface. Teleoconch shell fixed to hard (coral) surface, and growing as a cemented tube extending irregularly away from the larval shell without coiling. Teleoconch tube has narrow, close-spaced annular rings on the exposed tube surface. Annular rings vary slightly in width of the ring as tube diameter increases slowly during growth.

Remarks: The genus *Brazoverma* differs from other vermetiform gastropods in having a distinctly slender, high-spired larval shell and in having a cemented teleoconch tube with smooth-crested annular rings. There is very little variation in larval shells in the sample; they are all of the same size, dimensions, and ornamentation. There is no longitudinal ribbing on the teleoconch tube surface and no internal columnar laminae. The teleoconch tube has a diameter of 1–2 mm, smaller than most vermetid species, but that is consistent with growth onto the surface of small solitary corals. Teleoconch tube growth from the point of cemented fixation is primarily oriented toward the coral calice and does not wrap around the larval shell or coil. The growth direction is presumably a response to extend the tube upward across the coral substrate and away from or out of a nepheloid layer of bottom water containing suspended clay particles of the mud bottom environment.

Brazoverma has few characters in common with vermetiform fossil species known from Cenozoic deposits of the American Atlantic region: *Thylacodes* Guettard, 1770 (usually cited under the synonym names *Serpulorbis* Sasso, 1827, and *Lemintina* Risso, 1826), *Petaloconchus* Lea, 1843, and *Tripsycha* Keen, 1961, using the taxonomic summary of genera of Bieler and Petit (2011). *Thylacodes* is characterized by gregarious growth habit and has an irregularly coiled tube (Keen, 1961), *Petaloconchus* is characterized as a genus with two internal columnar laminae (Lea, 1846; Gould, 1994; Keen, 1961), and *Tripsycha* grows as a coiled cone (Keen, 1961; Garvie, 2021). Most fossil species in Cenozoic deposits of the Gulf of Mexico have teleoconch tubes with cancellate sculpture (Keen, 1961; Gardner, 1947; Garvie, 1996). The combination of distinctive larval shell, annular teleoconch ornamentation, and adaptation to coral substrate indicate the Little Brazos species is not closely related to other fossil species.

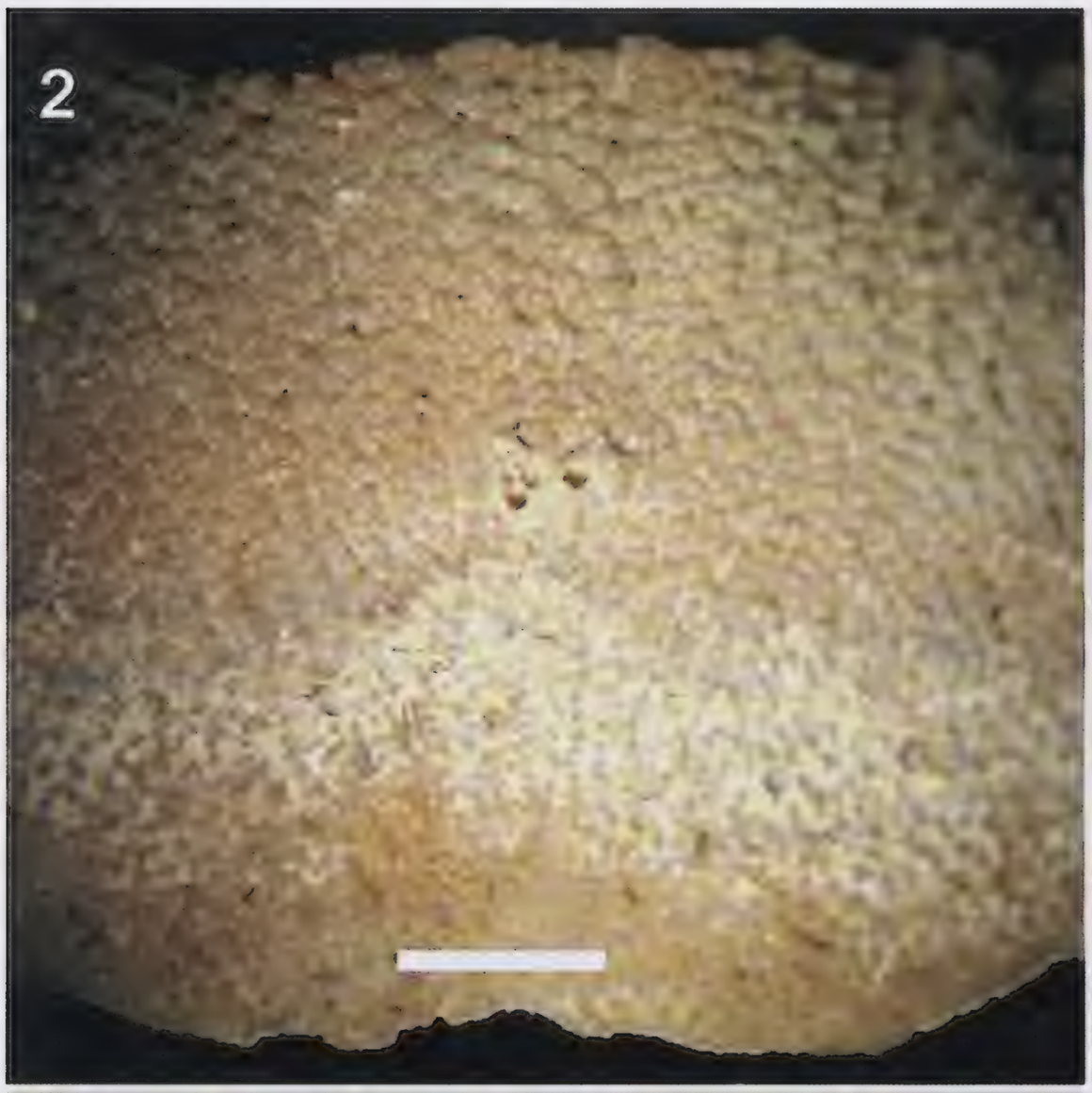
Etymology: An arbitrary combination of terms *brazo* for the Little Brazos River, collection site of the sample, and *verma* for the vermetiform growth habit. The name is formed with no connotations of gender.

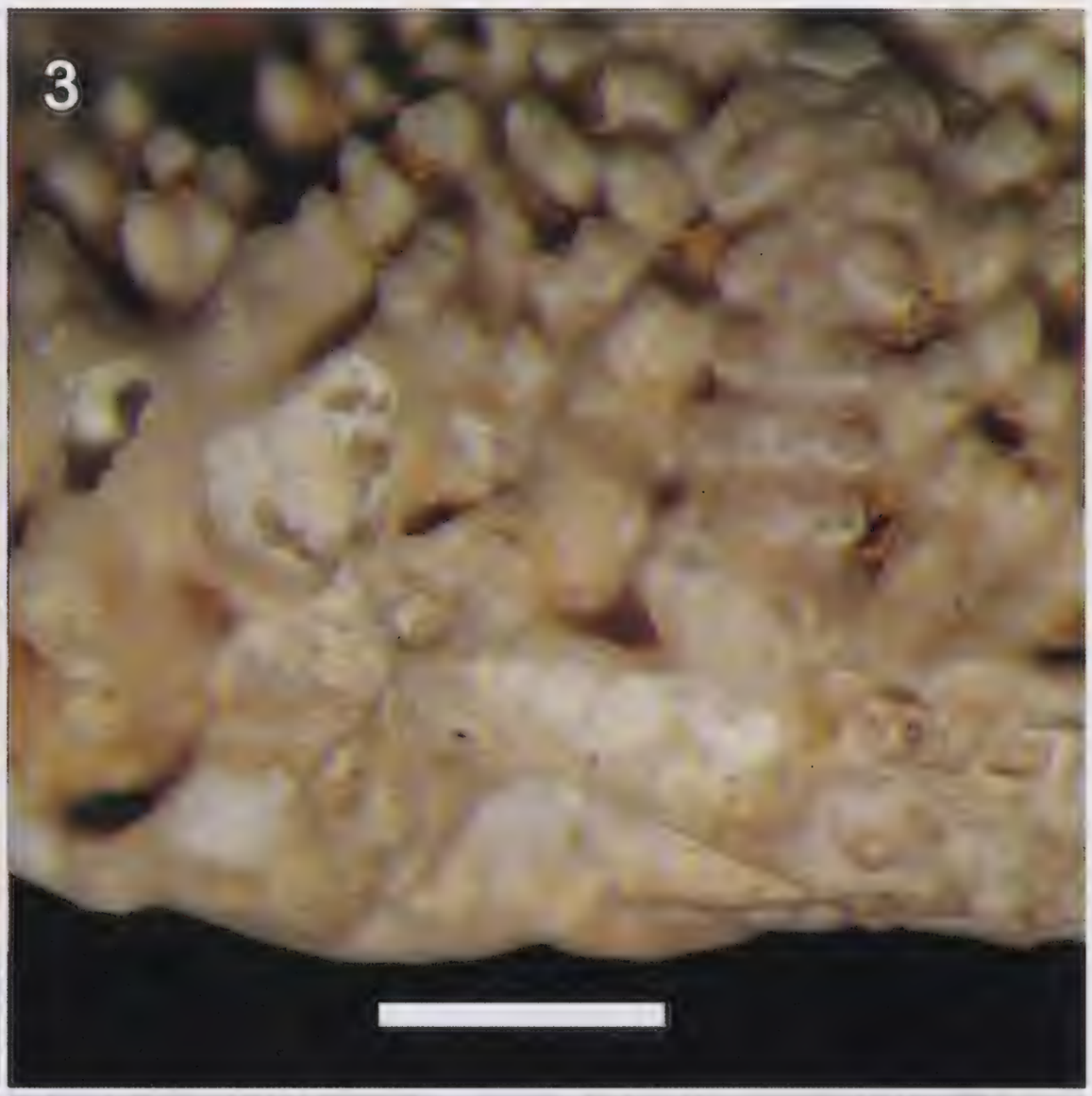
Brazoverma brazina new species
(Figures 2–11)

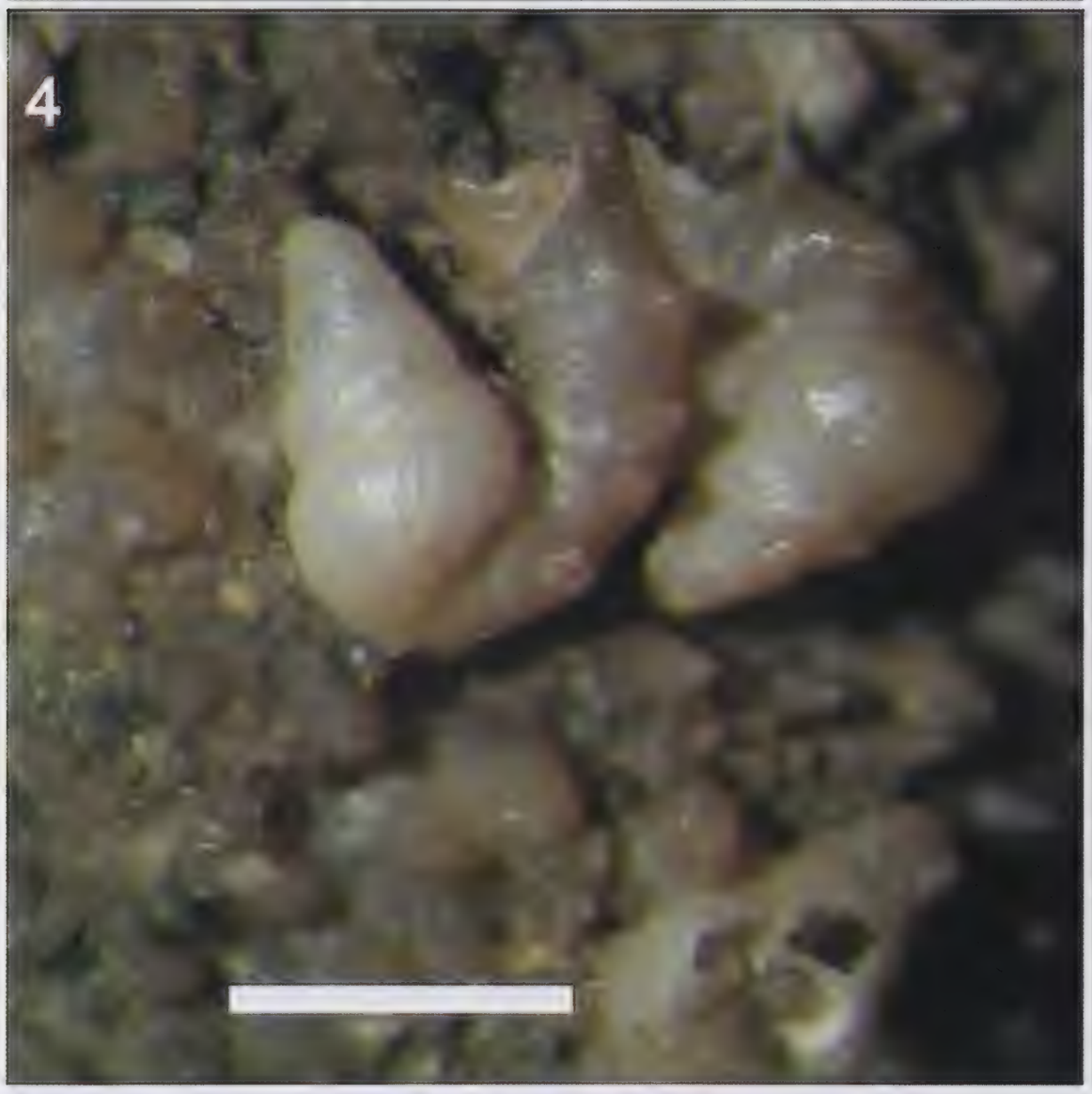
Diagnosis: Vermetiform gastropod with slender, high-spired, tight-coiled larval shell of 4–5 whorls ornamented with many close-spaced collabral ribs, teleoconch that does not wrap around the larval shell, and an irregular, uncoiled teleoconch shell with rounded, smooth-crested annular rings on the tube.

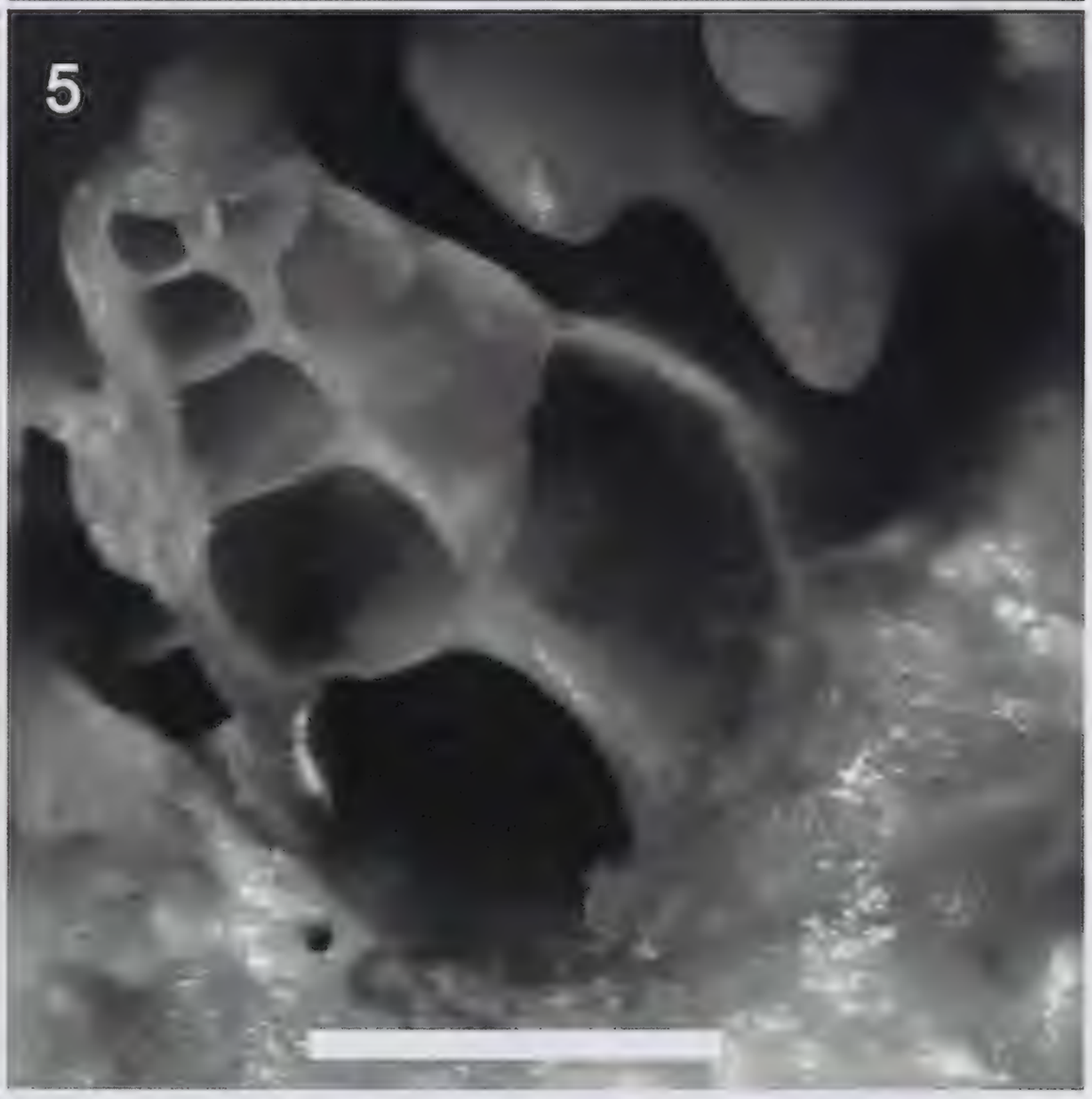
Description: Species with small larval shell (~1 mm length) of high-spiral, very uniform tight-coiled whorls covered the fine, close-spaced collabral ribs along 4–5 whorls below the tiny smooth hemisphere apical cap (Figures 2–5). The larval shell has a shiny surface where not overgrown by coral tissue. The collabral ribs have uniform width along the rib and are separated by narrow grooves from adjacent ribs. Larval shell attains 1 mm axial length and maximum 0.3 mm coil width and has a narrow, straight linear columella. The aperture margin present on larval whorls of the fossil is angled about 30° from the axis of coiling with the greatest projection of the margin at the anterior end of the aperture. The suture line between whorls is angled at ~ 75°–80° to axis of coiling.

Teleoconch tube growth to 3 cm long or more and reaching a 2 mm tube diameter (Figures 6–9). Teleoconch growth from the point of larval fixation begins with an abrupt change to irregular tube with no coiling or wrapping around the larval shell. Coiling of the first-formed teleoconch tube around the larval shell is common among other species of family Vermetidae (Keen, 1961; Savazzi, 1996; Golding et al., 2014), especially among species that retain some or major amounts of coiling. Tube growth of *B. brazina* extends toward the calice of the coral, either as a winding tube or in a nearly straight line with a gradually increasing diameter. There is no indication of excavation of the coral surface for attachment; an unnecessary action with corals that readily secrete coral tissue onto the surface of the gastropods, binding it in place. Tubes stay attached to the coral surface and do not project away







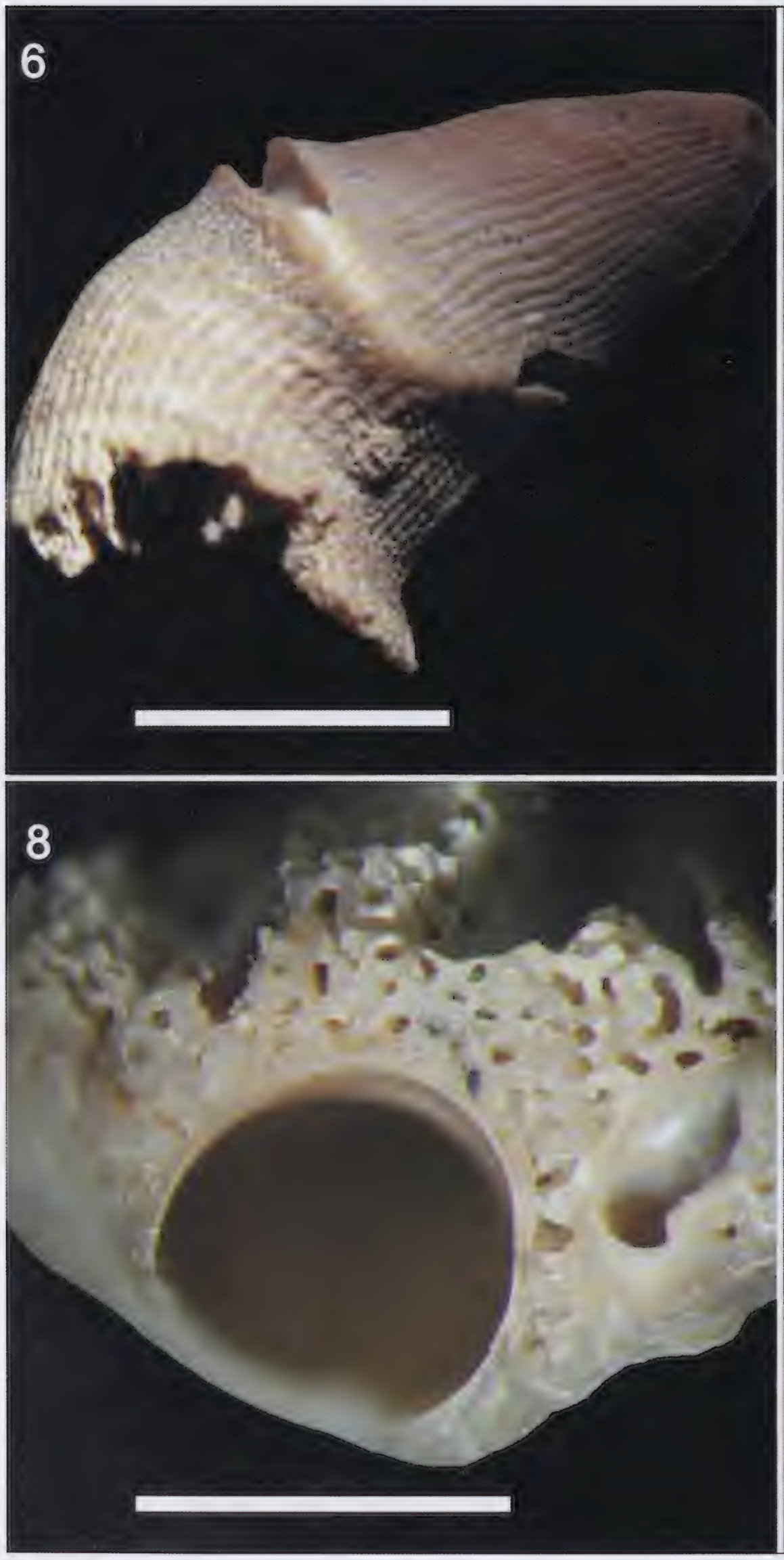


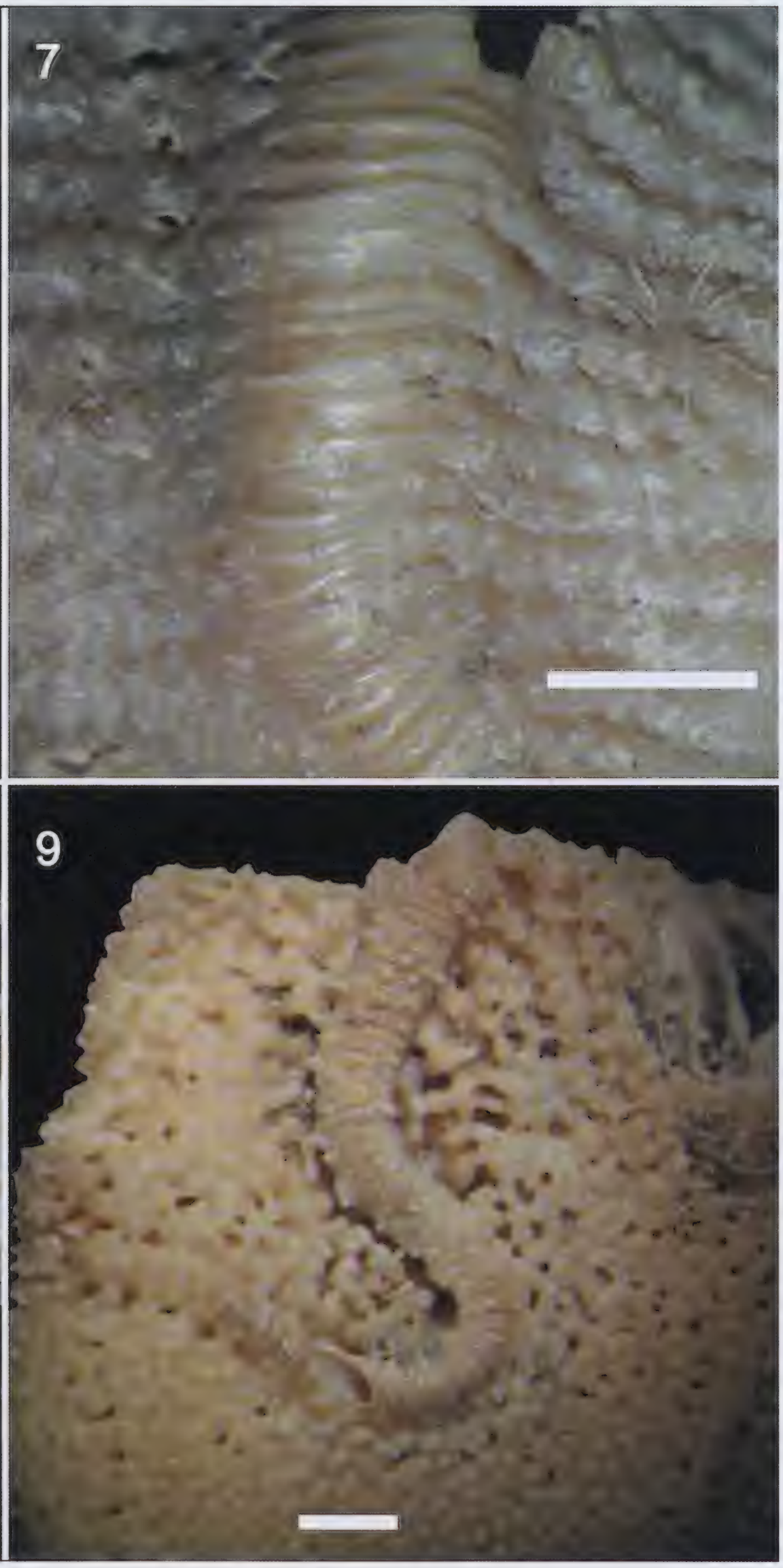
Figures 2–5. Protoconch. **2.** Specimen of free-living coral *Endopachys maclurii* shown in growth position with larval shell attached (center) above a white band indicative of depth of burial of the coral skeleton during life. Scale bar = 2 mm. **3.** Cluster of protoconchs attached to basal margin of coral skeleton. Scale bar = 1 mm. **4.** Cluster of protoconchs including holotype (center, NPL 001) with short section of teleoconch tube attached on coral surface, showing close-spaced collabral ribbing and shiny outer shell surface. The bumps present on earliest teleoconch tube are coral overgrowth knobs, distinguished from gastropod shell by size and color similar to surrounding coral surface. Scale bar = 1 mm. **5.** Interior view of a protoconch showing tight regular coiling of 5 whorls. The knob on apex of shell is part of the coral skeleton. Scale bar = 1mm.

from the coral surface except to project above the lip of a coral calice. There is no longitudinal ribbing or regular cancellate sculpture on the tube. The annular rings are semi-circular in cross section, are close-spaced, and vary slightly in width. They occur close together with only a groove between rings or have a narrow flat band of smooth tube between the rings. Where the tube bends some rings split along the crest into two rings midway across the tube. Some have minor bends, following minor changes in the shape of the tube aperture margin.

The teleoconch tube is thin-walled and constructed of irregular prisms aligned nearly perpendicular to the tube wall surface and semi-intergrown (Figure 11). The wall is often encrusted with a thin layer of coral skeletal tissue with a spongy texture, as shown by the continuity of a layer of spongy texture from coral to the outer tube wall (Figures 10, 11).

Remarks: The larval shell with its uniform tight coiled whorls and well-developed ornamentation is the most





Figures 6–9. Teleoconch. **6.** Tube of specimen (NPL 012) grown onto surface of solitary coral *Balanophyllia desmophyllum* with edges of tube overgrown by coral. Although the base of coral (upper right) shows attachment settlement scar, the mature coral lived as a free-living form held in place by partial burial. Scale bar = 1 cm. **7.** Detail of teleoconch tube (NPL 012) showing annular ribbing with smooth surface and minor variation in rib spacing and bending. Scale bar = 2 mm. **8.** View into interior of mature portion of teleoconch tube (NPL 012). Interior of tube is lined with a thin layer of irregular prismatic crystals. Scale bar = 2 mm. **9.** Early growth portion of teleoconch tube on coral *Endopachys maclurii*. Protoconch is attached on margin of coral, out of view. Annular ribbing is present, partly overgrown with scattered knobs of coral tissue. Scale bar = 1 mm.

distinguishing character of the fossil species. The larval whorls are so regular and well formed, including shiny outer surface and exact-aligned ribbing, that the shells would not be distinguished from tiny free-living gastropods if found as a loose, pre-attachment microfossil. The process of fixation on a hard surface and the abrupt change to growth as a winding tube reveals an adaptation of this species to vermetiform growth but the larval shell is unlike that of the protoconch of other vermetiform species.

The teleoconch shell is also a distinctive feature of this species. It is covered with annular rings with semi-circular cross section; they are a modified continuation of the collabral ribbing of the larval shell. Rings on the teleoconch increase in cross section width during growth and are less regularly spaced but are the dominant feature of the tube throughout growth. The teleoconch grows away from the larval shell, not wrapping around the apex of the shell.

Some areas of the teleoconch tube appear to have small nodes on the surface of the tube Figures 3, 4, 9), especially in areas where the outer surface of the tube is close to the level of surrounding coral surface. The inconsistency and irregularity of these nodes makes it unlikely that they are part of the teleoconch tube. Most of them are identical in size, shape, and coloration to the tops of nodes of the surrounding coral skeleton (Figure 4) and they appear to be produced by coral overgrowth onto the tube. Coral skeleton nodes have a darker honey-colored appearance than the plain white color of teleoconch tube. The nodes do not form in rows and do not form a cancellate pattern of noding characteristic of ornamentation on tubes of many fossil vermetiform species. Well-exposed, large-diameter portions of teleoconch tube are covered only with annular rings and lack nodes. Partial to extensive overgrowth of a film of coral skeletal tissue occurs on most of the specimens (Figures 6, 7, 10), including the interior surface of the tube of specimens that died while the coral was alive. The round nodes protruding as much as ¼ of the tube diameter above the teleoconch tube shown in Figure 4 have darker coloration than the tube skeleton and are identical to the tops of mounds of the adjoining coral skeleton. They are interpreted to be a covering of the teleoconch tube by the coral.

Type Material: Holotype NPL 00097417 (Figure 4), paratypes NPL 00097418-00097429 (Figures 2, 3, 5–11).

Type Locality: Route 21 bridge crossing of Little Brazos River, Brazos County, Texas, USA. 30.640994 N; –96.520838 W. The stratigraphic horizon containing fossils is also exposed on the stream bed of Little Brazos River at a position 180 m NNE of the highway bridge.

Distribution: Known only from the type locality.

Etymology: An arbitrary combination of terms *braz* and *ina* for the Little Brazos River, collection site of the

Figures 10–11. Microstructure of shell (NPL 014). **10.** Portion of teleoconch tube overgrown by thin layer of coral tissue on exterior and interior of tube, shown by continuity of spongy textured tissue from coral skeleton (upper right and lower left) and surface of tube. Scale bar = 200 µm. **11.** Enlargement of teleoconch wall structure showing wall composed of many bundles of fibrous crystals aligned perpendicular to wall surface. Scale bar = 20 µm.

sample producing specimens of the new species. Used as a name in apposition. The name is formed with no connotations of gender.

Substrate Preference of the Fossil Species: Specimens of the species described here were found only on the surfaces of small (1–2 cm long) solitary scleractinian corals. Bivalve and gastropod shells of comparable size to the corals and having solid smooth surfaces have no examples of encrustation by the new species. A large sample of fossiliferous mudstone containing the biological community was processed and all specimens larger than 0.5 mm were tabulated to species, providing comprehensive data on species composition of the biological community. The vermetid fossils occur mostly on the outer surface of *Endopachys maclurii* Lea, 1833 (to 1 cm height) and a few occur on the larger (2–3 cm height), less common coral *Balanophyllia desmophyllum* Milne-Edwards and Haime, 1848. Eleven of 100+ large (non-juvenile) specimens of *E. maclurii* are encrusted with juveniles of the fossil, some with as many as 8 individuals on a single coral specimen. Of 40 specimens of *B. desmophyllum* examined, only four have specimens of the fossil. However, the largest individual occurs on this coral species. 35 specimens of the vermetid fossil are available for study of which 95% are small, newly settled juveniles that died soon after settlement. Nearly all of them have some coral skeletal tissue overgrowing the tube, indicating settlement during life of the coral. No larval shell or teleoconch of the species was observed on the surface of the uncommon branching colonial coral *Archohelia singleyi* (Vaughan, 1900), also present in the fossil bed. No specimens were found on surfaces of the common large bivalves and gastropods or other taxa in the biological community. An examination of other large shells present, including *Venericardia (Venericor)*, *Turritella*, *Pseudoliva*, *Athleta*, and *Conus*, did not reveal any specimens of the new species. The beaks of *Venicardia (Venericor)* had larval settlers of corals and oysters but no settlers of *B. brazina*.

Both of the encrusted coral species are free-living species that lived with the basal surface buried in sediment to support themselves and maintain an upright position. Some coral specimens have a narrow ring of secondary carbonate coating the surface above the base of the coral. That appears to be a coating secreted by the coral at a level corresponding to the boundary between exposed surface and the base embedded within sediment. The coral *E. maclurii* has an irregular outer surface consisting of open meshwork, a "spongy" type of skeleton. The coral *B. desmophyllum* has a nearly solid outer surface with some openings in the outer wall. Skeletons of both corals appear to have had living soft tissue on the outer surface, pointing to an association of the new species with corals having soft tissue exposed on the outer wall during life.

It is noteworthy that *B. brazina* settled onto small corals living on a mud-dominated sea bottom, an area lacking long-lasting hard substrate. There is no indication of strong seafloor currents or waves disturbing the seafloor sediments. There are few shells or bioclasts larger than 5 cm in the sediment and most species of large size lived their lives partly or nearly buried within the mud. The only hard surfaces with fixed position and continually exposed during life are the small corals that probably had a short life span. A mud bottom substrate is an unlikely environment to have vermetiform gastropods present and the choice of small solitary corals as a host for settlement is unexpected. However, corals provided the most enduring hard exposed surface in that environment.

DISCUSSION

The distinctive teleoconch and protoconch of *B. brazina* make it easy to identify this gastropod. The low, arched, annular bands on the outer surface of the teleoconch tube distinguish this species from other fossil vermetiform gastropods. The only ones that come close are species of vermetid genera *Tripsycha* and *Petaloconchus* which have fine lines of circular banding (Keen, 1961), distinct from the annular bands of this new species. Not only are these much different in appearance, but the teleoconch of *Tripsycha* and *Petaloconchus* have a growth habit of conical coiling of early-formed portions of the teleoconch tube and would not be confused with *B. brazina*.

The protoconch of the new species differs substantially from other vermetiform species in having a high-spired, tight-coiled, well ornamented shell (Figures 4, 5), resembling the post-larval shell of some high spired gastropods. The ornament of *B. brazina* is the same along the length of the larval coil and does not increase in density or strength of ribbing along the coil. There is no indication of spiral ribbing or granular ornamentation. The extensive area of uniform fine ornamentation on the protoconch contrasts with the limited ornament or gradual change in ornament on most gastropod protoconchs. However, the size, 0.5–1.0 mm, is consistent with the size of gastropod protoconchs formed during larval growth from egg stage.

Available data does not allow determination if *B. brazina* embryos were released to planktotrophic life or grew primarily within an egg capsule, although the concentration of larval shells fixed on the lower edge of *Endopachys* corals (Figure 3) at what appears as a depth marker of coral base burial in sediment (Figure 2) suggests a free-living growth stage before fixation and the possibility of larvae crawling on mud substrate to locate an attachment site. Eight larval shells are attached to the wedge-shaped base of one *Endopachys* coral and a majority of larval shells attached at a similar position.

The small size of *B. brazina* is consistent with growth on small solitary corals that inhabit a mud-bottom sea floor, but adaptation to encrusting small corals with living tissue in that environmental setting is quite unusual. There is no evidence of encrustation onto shell surfaces that lack a cover of living tissue and no evidence of the

animal excavating the substrate surface for a place to attach. Settlement occurred on the high points of nodes on the coral surface and remained perched above the main mass of coral skeleton. Overgrowth by the coral occurred soon after settlement and the overgrowth provided for secure attachment of the gastropod to its substrate. It does not seem coincidence that most settlement occurred on the coral species with the most irregular surface.

Relationship to Other Vermetiform Species: Gastropod protoconchs are generally considered to be conservative characters and are useful for determining gastropod relationships, including relationship of vermetid gastropod species (Golding et al., 2014). The Eocene fossil *B. brazina* has a distinctive protoconch typical of gastropods but the extended and consistent close-coiled, helical, logarithmic growth and non-varying collabral ribbing is much different from the protoconchs of other vermetiform gastropods. It does not provide indication of relationship to the three main vermetiform gastropod groups: Vermetidae, Siliquariidae, or *Vermicularia* of the Turritellidae. Species of *Vermicularia* (Bieler and Hadfield, 1990; Savazzi, 1996; pers. obs. of *Vermcularia sp.*) and species of the Siliquariidae (Savazzi, 1996; Gould, 1996; Bieler, 2004; Bieler and Simone, 2005; pers. obs. of *Tenagodus barbadensis*) have protoconchs with gradual change in coiling and serial change in ornamentation of the protoconch. Those species also have gradual change into irregular uncoiled teleoconch growth. This contrasts with the abrupt, major change in growth pattern from protoconch to teleoconch of *B. brazina* at the time of fixation to a hard surface. There is no evidence suggesting relationship of *B. brazina* to the Siliquariidae or the Turritellidae.

The abrupt change in growth pattern at the time of fixation to a hard surface of *B. brazina* is a character similar to that of vermetid gastropods, but it is also a character of serpulid polychaetes and is a consequence of shell fixation, and therefore environmentally controlled (Savazzi, 1996). Similarly, species of *Vermicularia* and of siliquariids are associated with life on or embedded in sponges and their teleoconch growth is as environmentally influenced as much as that of fixation to a hard substrate. Teleoconch characters insulated from environmental control include features of tube ornamentation and shell microstructure. The tube ornamentation of *B. brazina* differs from other vermetiform species in consisting solely of annular rings with a low arched cross section and no indication of longitudinal ribbing or nodes. The shell microstructure of *B. brazina* teleoconch tube consists of irregular prisms (Figure 11), different from the multi-layered shell wall present in vermetid gastropods (Keen, 1961), although imaging of protoconch shell microstructure is needed to determine if it remains constant from protoconch to teleoconch. This combination of protoconch contrasting with other vermetid species, distinct teleoconch ornamentation, and unusual shell microstructure suggests distant relationship of *B. brazina* to vermetid gastropods and the possibility of independent origin for the species.

An evaluation of possible ancestors for *B. brazina* has to focus on preserved shell characters and growth patterns that provide data for comparison. A survey of protoconchs on gastropods in the Eocene fossil sample and of living caenogastropods revealed no close obvious relationships to other gastropods but did find some similarity to protoconchs of the Epitoniidae. This stimulated a comparison of *B. brazina* with species of epitoniids because of the strong association of epitoniids with life on living corals, either as commensals, micropredators, or ectoparasites (Gittenberger and Hoeksema, 2013). This association is strikingly similar to the known coral association of *B. brazina*, that is inferred to have settled only onto the surface of corals with living soft tissue. This provides good reason for considering an origin for *B. brazina* from ancestors different than those of vermetid gastropods. Transition from commensal life on coral tissue to fixed attachment to soft tissue-covered coral surface is potentially a simple change that would lead to other character changes.

Although an origin from epitoniids is not clearly demonstrated (the protoconch of an *Epitonium* species illustrated by Gittenberger (2003) is mostly smooth), the earliest post-larval shell of the *Epitonium* species illustrated by Gittenberger and Hoeksema (2013) is very similar to the larval shell of *B. brazina* and collabral ornamentation is the norm for epitoniids. A miniaturization of the epitoniid shell could produce a pre-fixation shell very much like that *B. brazina*. A growth history that links *B. brazina* to epitoniids is the deduction that *B. brazina* larvae settled primarily or entirely on corals with soft tissue covering the skeleton surface. Observation on the larval development of *Epitonium albidum* (Robertson, 1983) reveal a substantial interval of planktotrophic life during protoconch growth before settling onto a surface and metamorphosis. This larval history matches the inferred larval life history of *B. brazina* determined from the clustering of larval shells on or near the base of Eocene *Endopachys* corals.

The hypothesis of origin for the new species from an epitoniid ancestor cannot be tested with available data, but the known association of epitoniids with corals makes a connection likely. An assignment to family is not made in this report, pending more data needed to justify making a family rank. Other Eocene examples of the new species need to be located to further document this type of worm snail and data on protoconchs of other fossil species is needed for comparison. The oldest reliably known vermetid is of Cretaceous age (Bieler and Petit, 2011) so the descent from an ancestor should be determinable with study of similar well-preserved fossils.

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New species of *Aldisa* Bergh, 1878 (Gastropoda: Nudibranchia: Cadlinidae) from New Caledonia (western Pacific) and Reunion (Indian Ocean)

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ABSTRACT

Two new species of *Aldisa* are described based on specimens collected from New Caledonia, Western Pacific and Reunion, Indian Ocean. These two new species have a bluish background color similar to other species of *Aldisa* primarily known from the tropical Indo-Pacific. However, the two new species are distinguishable from other know species and from each other by several external and internal morphological characteristics. The two new species are also characterized using partial sequence data from the COI mitochondrial gene.

Additional Keywords: Heterobranchia, COI, phylogenetics

INTRODUCTION

The genus *Aldisa* Bergh, 1878 contains 20 valid species distributed across tropical, temperate, and cold waters around the world (Tibiriçá et al., 2019). Members of *Aldisa* are characterized by an unusual radular morphology with extremely elongate and thin radular teeth, among other characteristics (Millen and Gosliner, 1985). The taxonomic affinities of *Aldisa* have been controversial, while morphological phylogenetic analyses place it among the family Doridiidae (Valdés, 2002), molecular phylogenies confirmed its sister relationships with *Cadlina* (Johnson, 2011). There are two distinct body types among members of *Aldisa*, some species are red (or white) in color, sometimes with other pigmentation, often with depressions, ridges and/or conical tubercles, whereas other species are blue or bluish-grey often with black and yellow-orange lines, and large, low, round, simple tubercles. While the red group is widespread across ocean basins and temperature gradients (Millen and Gosliner, 1985; Tibiriçá et al., 2019), the bluish-grey group is restricted to the tropical Indo-Pacific (Elwood et al., 2000; Perrone, 2001; Gosliner and Behrens, 2004) and West Africa (Ortea and Ballesteros, 1989). It is unclear whether these two groups are monophyletic, as sequence data for many species of *Aldisa* are lacking.

The goal of this paper is to describe two new species of the *Aldisa* group recently discovered in the Indian and Western Pacific Oceans. These two species have a bluish background color, similar to that of some other species previously described from the Indo-Pacific region (see Elwood et al., 2000; Perrone, 2001; Gosliner and Behrens, 2004), but are morphologically distinct. The two new species are characterized using sequence data from the COI mitochondrial gene and a preliminary phylogenetic analysis with other species of *Aldisa* is provided.

MATERIALS AND METHODS

Collection and Preservation: The specimen from Reunion was collected during the MesoRun program by rebreathing SCUBA diving between 70–100 m deep as part of an effort to document the mesophotic fauna of this island. The specimens from New Caledonia were collected by SCUBA diving around the Nouméa area, between 5–7 m depth. All specimens were photographed alive, preserved in ethanol 90%, and deposited at the Museum National d'Histoire Naturelle, Paris (MNHN).

DNA Extraction, PCR, and Molecular Analyses: DNA extraction was performed using a hot Chelex® protocol with approximately 1-3 mg of the foot cut into fine pieces. The tissue pieces were then placed in a 1.7mL microcentrifuge tube containing a 10% Chelex® 100 (Bio-Rad) in TE buffer. Four µl of 20mg/ml Proteinase K was added and the samples were incubated for 20 minutes in a 56°C water bath with intermittent vortexing. Samples were transferred to a 100°C heat block for eight minutes and then centrifuged at >10,000 × g for three minutes. The supernatant was transferred to a new tube and used for PCR reactions.

The universal CO1 primers (LCO1490 5′-GGTCAA CAAATCATAAAGATATTGG-3′, HCO2198, 5′-TAAAC TTCAGGGTGACCAAAAAATCA-3′ developed by Folmer et al., 1994) were used to amplify the regions of interest for all specimens. Each PCR reaction consisted of 38.50 µL H_2O, 5.00 µL 10× DreamTaq Buffer (Thermo Fisher Scientific Baltics, Vilnius, Lithuania), 1.25 µL BSA (Bovine Serum Albumin, Thermo Fisher Scientific Baltics, Vilnius, Lithuania), 1.00 µL 10mM dNTPs, 1.00 µL 10 mM primer 1, 1.00 µL primer 2, 0.25 µL 5 mg/mL Taq, and 2.00 µL extracted DNA. Reaction conditions were an initial denaturation for 3 min at 95°C, 35 cycles of 1) denaturation for 45 sec at 94°C, 2) annealing for 45 sec at 45°C, and 3) elongation for 2 min at 72°C, and a final elongation for 10 min at 72°C.

PCR products yielding bands of appropriate size (approximately 700 bp) were purified using the The E.Z.N.A.® Cycle-Pure Kit Purification Kit (Omega Bio-tek, Inc., Norcross, GA, USA). Cleaned PCR samples were quantified using a NanoDrop 1000 Spectrophotometer (Thermo Scientific). Samples were sequenced at Retrogen Inc (San Diego, CA, USA). The sequences were deposited in GenBank with accession numbers.

For the phylogenetic analyses, partial COI (cytochrome oxidase subunit I) sequences of the following species were obtained from GenBank (in alphabetical order): *Aldisa albatrossae* Elwood, Valdés, and Gosliner, 2000 (voucher CASIZ 181288, accession # KP871632.1), *Aldisa banyulensis* Pruvot-Fol, 1951 (accession # AY345039.1), *Aldisa fragaria* Tibiriçá, Pola and Cervera, 2017 (vouchers MB28-004392–3, accession # MF288003–4), *Aldisa sanguinea* (J. G. Cooper, 1863) (voucher CASIZ 182031, accession # MF958435), *Aldisa smaragdina* Ortea, Pérez and Llera, 1982 (isolate MM23, accession # KF992175), *Aldisa* sp. (voucher CASIZ175733, accession # MF958436), *Aldisa zavorensis* Tibiriçá, Pola, and Cervera, 2017 (voucher MB28-004397, accession # MF288005), and *Aldisa zetlandica* (Alder and Hancock, 1854) (isolate OS3, accession # KU695603). The species *Cadlina rumia* Er. Marcus, 1955 (CASIZ 175456) was selected as the outgroup based on recent phylogenetic analyses confirming the sister relationship between *Cadlina* and *Aldisa* (Johnson, 2011). Sequences for each gene were assembled and edited using Geneious Prime® 2019.2.3 (Kearse et al., 2012). Geneious was also used to check for open reading frames, check for contamination using the BLAST plugin, extract the consensus sequences between the primer regions, construct the COI alignment using the default parameters, and calculate pairwise identity percentages. The sequences were trimmed after alignment to 633 bp.

The phylogenetic analyses were conducted for the COI partial sequences. The Akaike information criterion (Akaike, 1974) was executed in JModelTest 2.1.7 (Darriba et al., 2012) to determine the best-fit models of evolution (GTR+I+G). The Bayesian analysis was executed in MrBayes 3.1.2 (Huelsenbeck and Rannala, 2004), partitioned by gene (unlinked). The Markov chain Monte Carlo analysis was run with two runs of six chains for ten million generations, with sampling every 100 generations. The default 25% burn-in was applied before constructing majority-rule consensus tree/s. The maximum likelihood analysis was conducted with RaXMLGUI 2.0.0-beta11 (Silvestro and Michalak, 2012) using the GAMMAGI model with 10,000 bootstrap repetitions. Nodes in the resulting phylogenetic tree with Posterior probabilities (PP) ≥ 90% and bootstraps values (MLB) ≥ 70% were interpreted as supported.

Morphological Examination: Preserved specimens were dissected and the internal features were examined using a dissecting microscope. The buccal mass of one individual of each species was removed and dissolved in 10% sodium hydroxide until the radula and jaw were isolated from the surrounding tissue. The radula and jaw were then rinsed in water, dried, mounted and sputter coated for examination with a scanning electron microscope (SEM) Jeol JSM-6010 SEM at the California State Polytechnic University, Pomona.

RESULTS

Molecular Phylogenetic Analyses: Bayesian and Maximum Likelihood analyses of the COI mitochondrial gene dataset yielded trees with the same overall topology (Figure 1). In both trees, the species *A. zetlandica* and *A. sanguinea* from cold and temperate waters in the Atlantic and Eastern Pacific respectively represent the earliest diverging lineages, but those positions are only supported in the Bayesian analysis. Among the rest of the species, the blue or bluish-grey species are monophyletic (PP = 1, MLB = 100), as well as the red species, excluding *A. sanguinea* (PP = 1, MLB = 100). The red species clade has two subclades, one containing Indo-Pacific taxa (PP = 1, MLB = 98), including *A. zavorensis*, *A. fragaria*, and an unidentified species from Malaysia, and the other Atlantic species (PP = 1, MLB = 100), including *A. banyulensis* and *A. smargdina*. The relationships among the three blue or bluish-grey species (*A. albatrossae* and the two new species described below) were not well resolved due to low support values.

Morphological Examination: The morphological characteristics of the specimens examined revealed consistent differences between them and with other species of *Aldisa* previously described. These differences are described and discussed below in the systematics section.

SYSTEMATICS

Aldisa theveneti new species

(Figs. 2–4, 6–7, 10–13)

Aldisa sp. 1 Gosliner et al. 2018: 88.

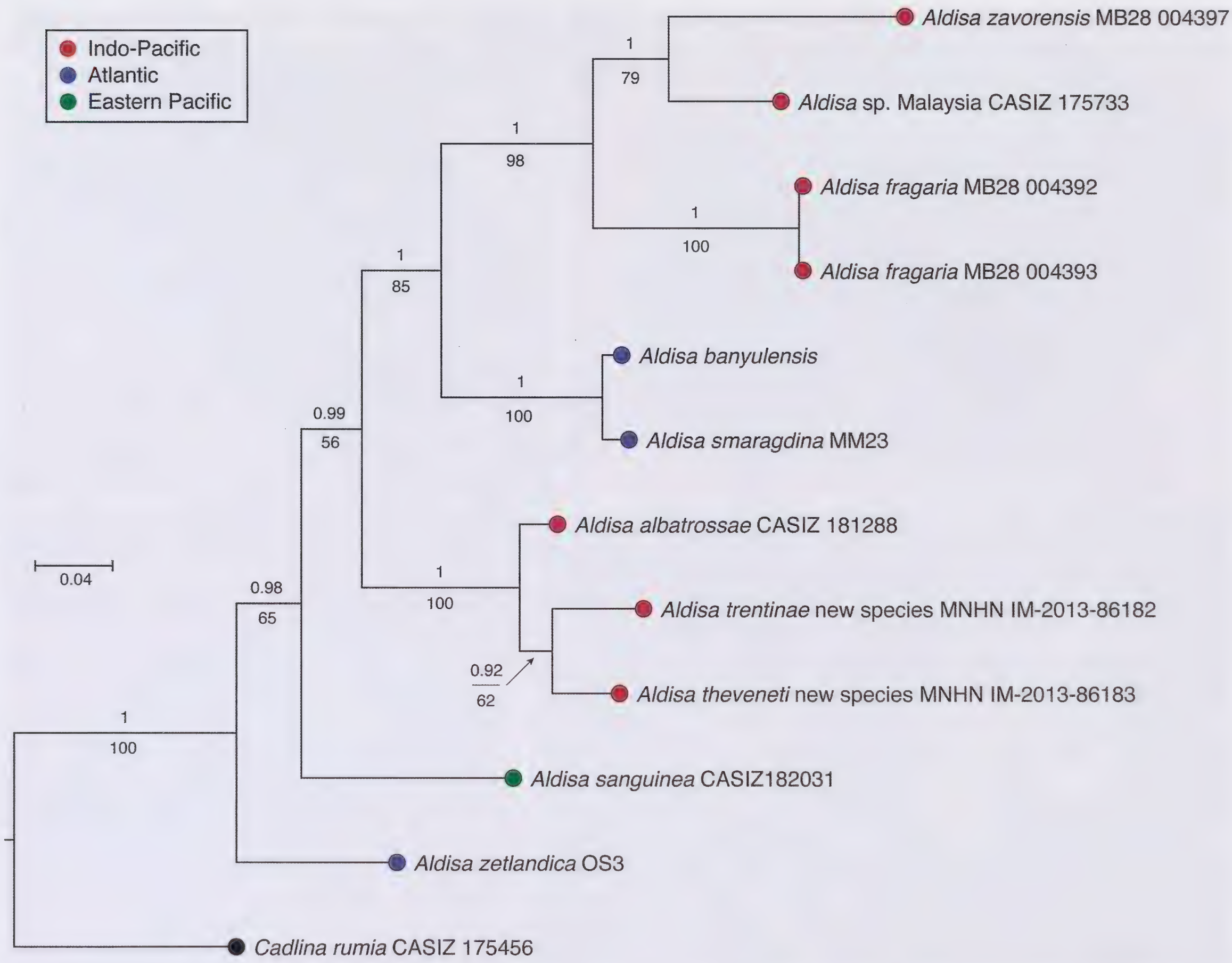


Figure 1. Bayesian consensus phylogenetic tree of the COI sequence data including posterior probabilities (PP) from the Bayesian analysis and bootstrap values from the maximum likelihood (ML) analysis. Geographic range of the species examined is color coded as indicated in the legend.

Description

Living animals 5–15 mm in length. Dorsum bluish (Fig. 2–4), darker at edges, lighter towards center of body, covered by numerous, low, conical tubercles. Tubercles with bluish-white tips radiating bluish-white lines, anastomosing with lines from other tubercles, forming complex network. Two black patches, longitudinal patch between the rhinophores, often bifurcating posteriorly (Fig. 2), and thin, transverse patch anterior to gill, present in all specimens, but faint, almost invisible in some animals (Fig. 3–4). At least three orange patches or lines also present in all specimens, "H"-shaped patch anterior to rhinophores, transverse line posterior to rhinophores, extending into mantle margin, and arch anterior to gill, almost reaching posterior end of body; some specimens may have additional orange patches on dorsum. Rhinophores conical, elongate, uniformly pale blue to white, with 5–7 lamellae. Three bipinnate branchial leaves with same color as rhinophores, conical, elongate; anterior leaf much longer than other two. Anterior portion of foot grooved but not notched. Head simple, with no oral protrusions (Fig. 10).

Radular formula is undeterminable owing to large number of elongate, overlapping teeth. Radular teeth narrow, elongate with broad triangular bases (Fig. 6–7). Top third or fourth of each tooth bears 12–18 sharp denticles, extending along the side of each tooth.

Denticles on apex much longer than those on sides, pointing towards base of teeth (Fig. 6). Reproductive system triaulic. Ampulla tubular, wide, narrowing into thin tube that connects with prostate before entering female gland complex (Fig. 11). Prostate granular, tubular, four times as long as ampulla, narrowing distally into thin duct that expands into wide deferent duct. Deferent duct merges with vagina at short genital atrium. Penis armed with triangular to elongate hooks (Fig. 13). Vaginal duct much narrower than deferent duct, connecting to irregular bursa copulatrix. Another duct connects bursa copulatrix to much smaller, elongate seminal receptacle (Fig. 12). Short uterine duct emerges from middle of this duct and connects into female gland.

Type Material: Holotype: 8 October 2018, 6 mm long, from type locality (MNHN IM-2013-86185).

Type Locality: Baie de Magenta, Nouméa, New Caledonia, 5 m depth.

Additional Material Examined: Baie de Magenta, Nouméa, New Caledonia, 8 Oct 2018, 5 m depth, 1 specimen 5 mm long (MNHN IM-2013-86184); Mont-Dore, New Caledonia, 8 October 2019, 7 m depth, 1 specimen 12 mm long (MNHN IM-2013-86183).

Etymology: This species is named after Yves Thévenet from Nouméa, New Caledonia, avid and passionate sea slug collector, who found this species alive for the first time.

Geographic Range: *Aldisa theveneti* new species appears to be endemic to New Caledonia.

***Aldisa trentinae* new species**
(Figures 5, 8–9, 14–16)

Description: Living animal 10 mm in length. Dorsum bluish (Figure 5), darker at edges, lighter towards center of body, covered by numerous, low, conical tubercles. Tubercles with whitish tips radiating whitish lines, anastomosing with lines from other tubercles, forming complex network. Several yellowish-orange patches on dorsum, two small irregular dots anterior to rhinophores (Figure 5), a "V"-shaped transverse patch posterior to rhinophores, extending into mantle margin, and arch anterior to gill, almost reaching posterior end of body. Rhinophores tentacular, elongate, uniformly white, with 11 lamellae. Six bipinnate branchial leaves with same color as rhinophores, short; all leaves similar in size. Anterior portion of foot grooved but not notched. Head simple, with no oral protrusions (Figure 14).

Radular formula is undeterminable owing to large number of elongate, overlapping teeth. Radular teeth narrow, elongate with broad triangular bases (Figures 8–9). Top third or fourth of each tooth bears 12–16 sharp denticles, extending along the side of each tooth. Denticles on apex much longer than those on sides, pointing towards base of teeth (Figure 8). Reproductive system triaulic. Ampulla tubular, wide, narrowing into thin tube that connects with prostate before entering female gland complex (Figure 15). Prostate granular, tubular, twice as long as ampulla, narrowing distally into thin duct that expands into wide deferent duct. Deferent duct merges with vagina at short genital atrium. Penis armed with hooks of various shapes (Figure 16). Vaginal duct twice narrower than deferent duct, connecting to spherical bursa copulatrix. Another duct connects bursa copulatrix to slightly smaller, oval seminal receptacle. Short uterine duct emerges from middle of this duct and connects into female gland.

Type Material: Holotype: 3 November 2020, 10 mm long, from type locality (MNHN IM-2013-86182).

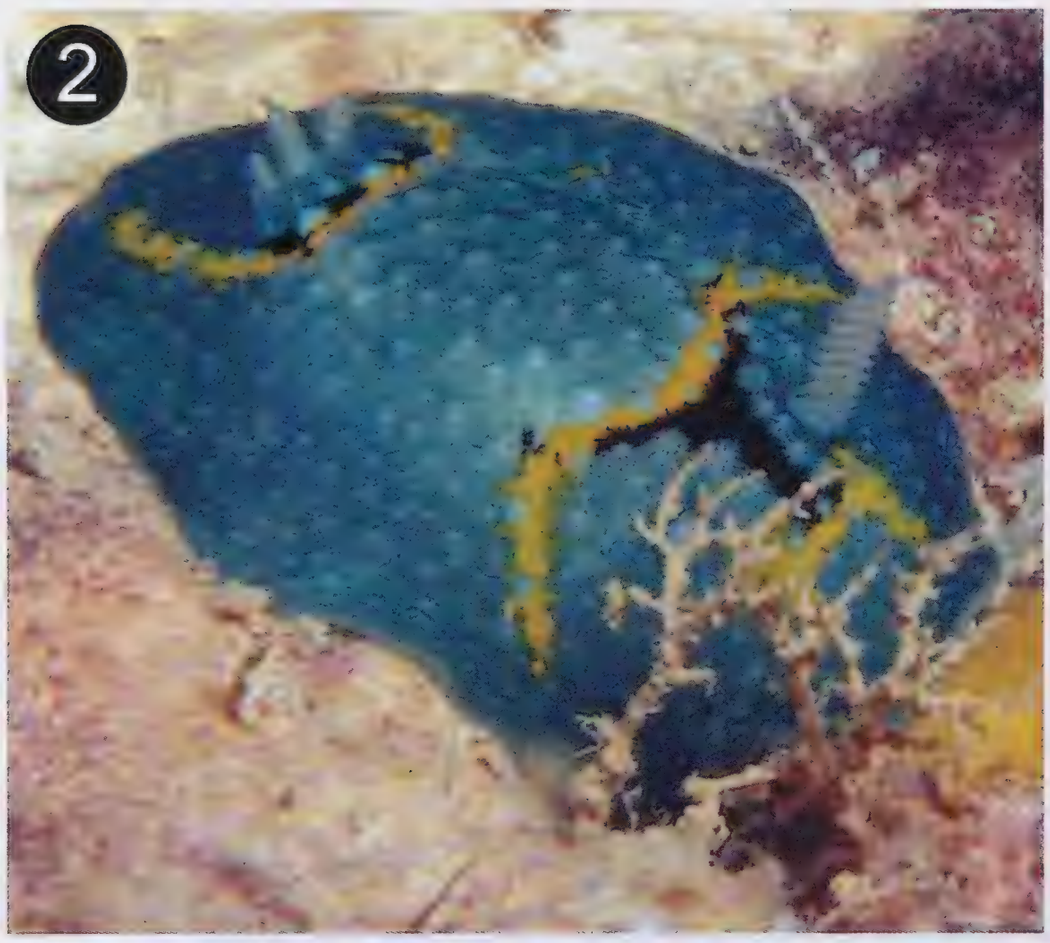
Type Locality: St. Leu, Reunion, 88 m depth.

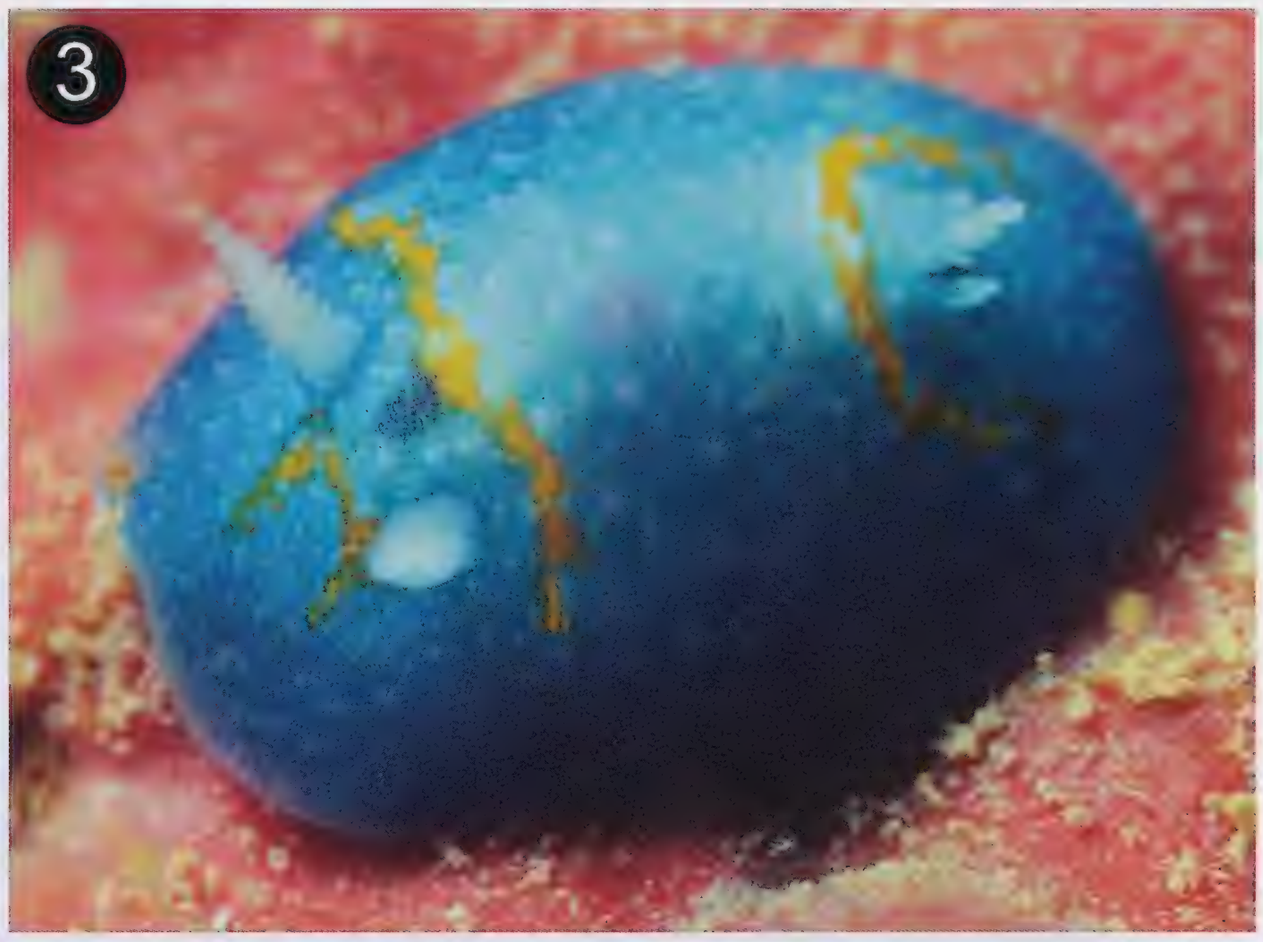
Etymology: This species is named after Dr. Florence Trentin of the Université de La Réunion for providing the holotype for study.

Geographic Range: *Aldisa trentinae* new species appears to be endemic to Reunion.

DISCUSSION

While more species of *Aldisa* have either a white or a red background color, sometimes with other pigmentation, several species possess a bluish-grey background color with black and yellow-orange lines, similar to *A. theveneti* new species and *Aldisa trentinae* new species. These include the Indo-Pacific species *Aldisa albatrossae* Elwood, Valdés, and Gosliner, 2000, *A. williamsi* Elwood, Valdés and Gosliner, 2000, *A. erwinkoehleri* Perrone, 2001, and *A. andersoni* Gosliner and Behrens, 2004 (see Elwood et al., 2000; Perrone, 2001; Gosliner and Behrens, 2004). However, all these species have prominent white, rounded tubercles and longitudinal black lines, very different from the small, bluish tubercles and the short black line restricted to the area between the rhinophores of *A. theveneti* new species, or the bluish tubercles with no black lines of *A. trentinae* new species. Among the species listed above, only *A. albatrossae* has a pattern of black and orange pigment similar to that of *A. theveneti* new species and *A. trentinae* new species. But, in addition to the size and color of the dorsal tubercles, *A. albatrossae* differs from *A. theveneti* new species and *A. trentinae* new species by the fact that *A. albatrossae* often lacks orange pigment anterior to the rhinophores and the posterior orange patch never forms a complete arch (see Elwood et al., 2000) as in *A. theveneti* new species and *A. trentinae* new species. Internally, *A. albatrossae* is characterized by having a hook-shaped radular tooth also present in *A. williamsi*, that is absent in both *A. theveneti* new species and *A. trentinae* new species; in addition, the penial spines of *A. albatrossae* are much more triangular than in *A. theveneti* new species and *A. trentinae* new species, clearly differentiating these species.

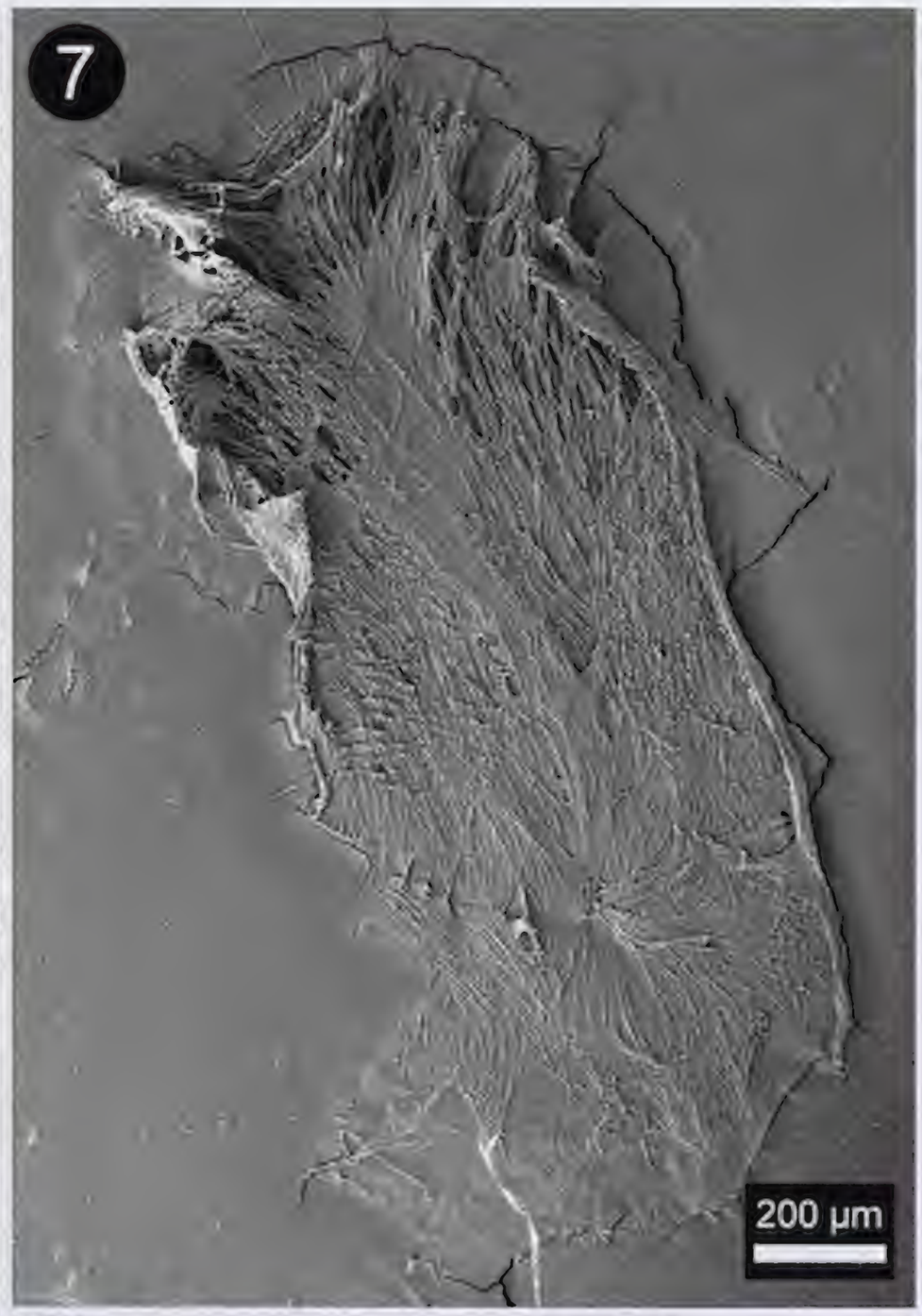


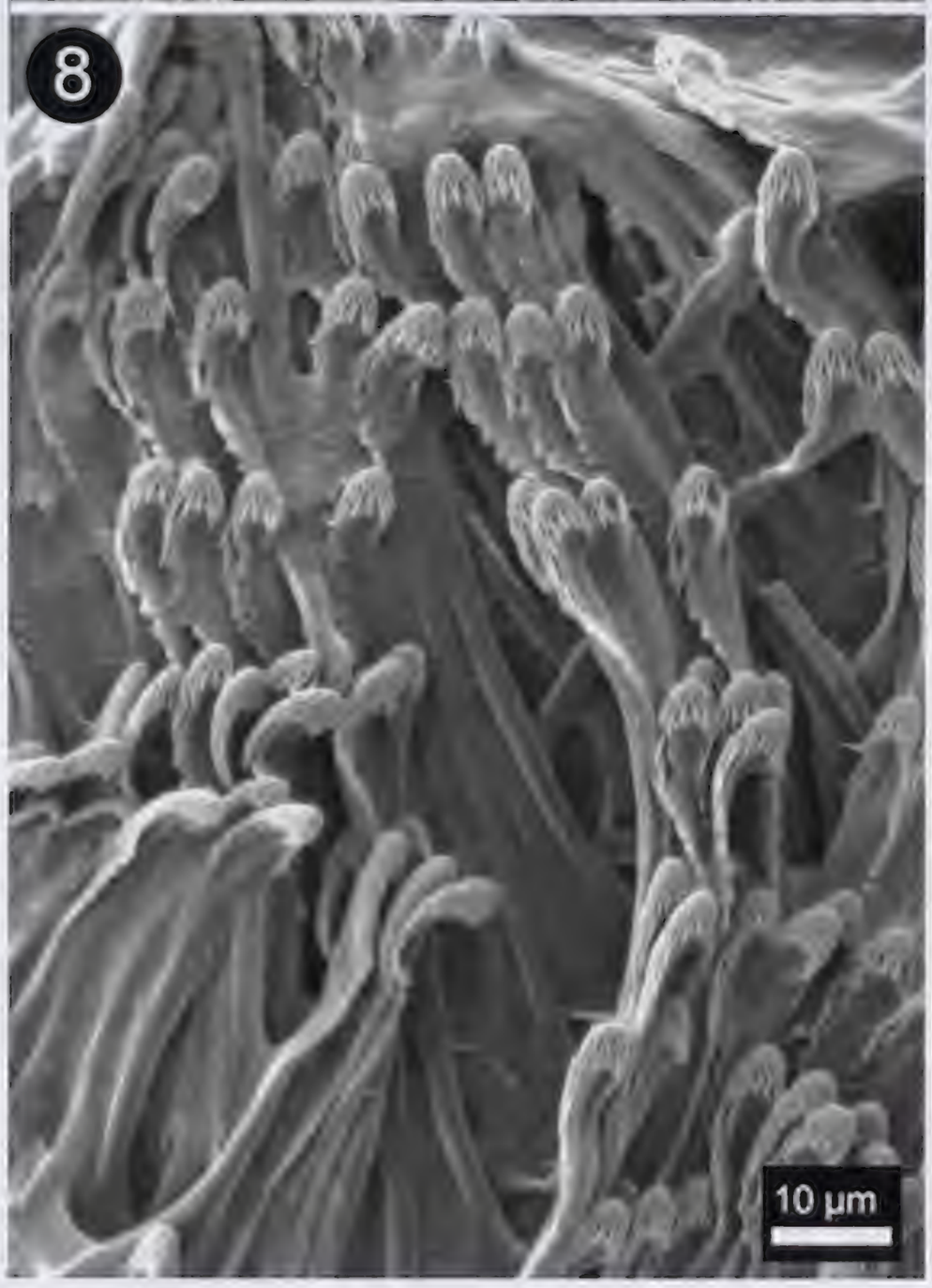


Figures 2–5. Photographs of live animals of ***Aldisa theveneti* new species** and ***A. trentinae* new species**. **2.** Dorsal view of a specimen of ***A. theveneti* new species** from Mont-Dore, New Caledonia (MNHN IM-2013-86183). **3.** Dorsal view of the holotype of ***A. theveneti* new species** from Baie de Magenta, Nouméa, New Caledonia (MNHN IM-2013-86185). **4.** Lateral view of a specimen of ***A. theveneti* new species** from Baie de Magenta, Nouméa, New Caledonia (MNHN IM-2013-86184). **5.** Dorsal view of the holotype of ***A. trentinae* new species** from St. Leu, Reunion (MNHN IM-2013-86182).

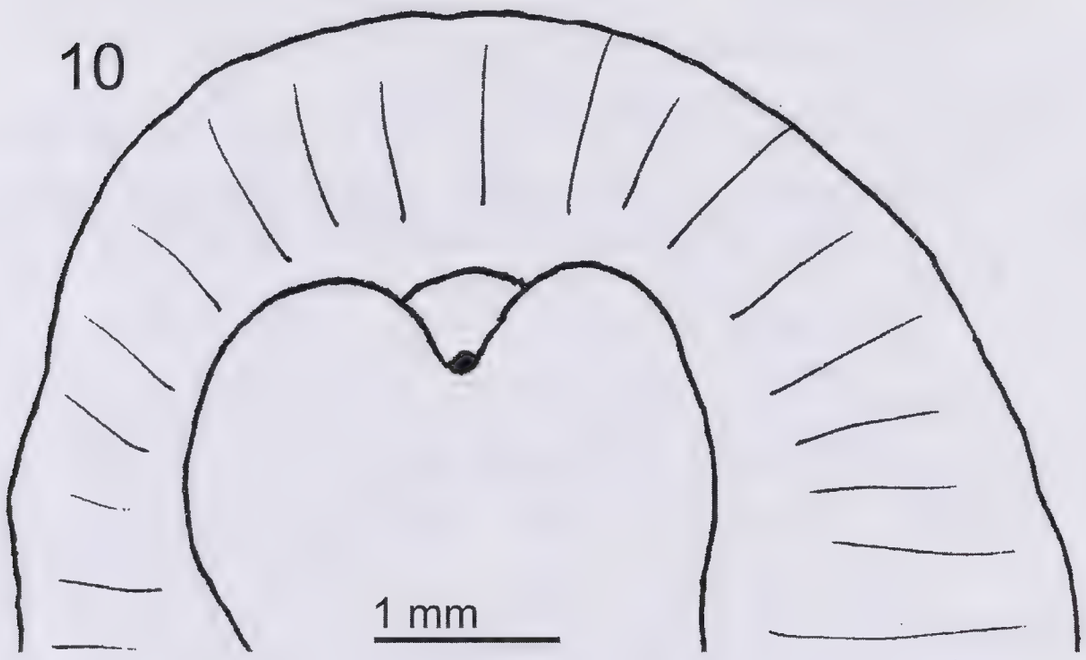
Aldisa theveneti new species differs from *A. trentinae* new species in several regards. Externally, *A. trentinae* new species does not have black pigment, the yellow-orange lines are more diffuse and cover larger areas than in *A. theveneti* new species. Internally, *A. theveneti* new species is clearly distinct from *A. trentinae* new species in several regards; in *A. theveneti* new species the bursa copulatrix is irregular in shape and much larger than the elongate seminal receptacle, while in *A. trentinae* new species both the bursal copulatrix and the seminal receptacle are oval and the former is slightly larger than the latter. Moreover, the prostate of *A. theveneti* new species is longer and more convoluted than in *A. trentinae* new species.

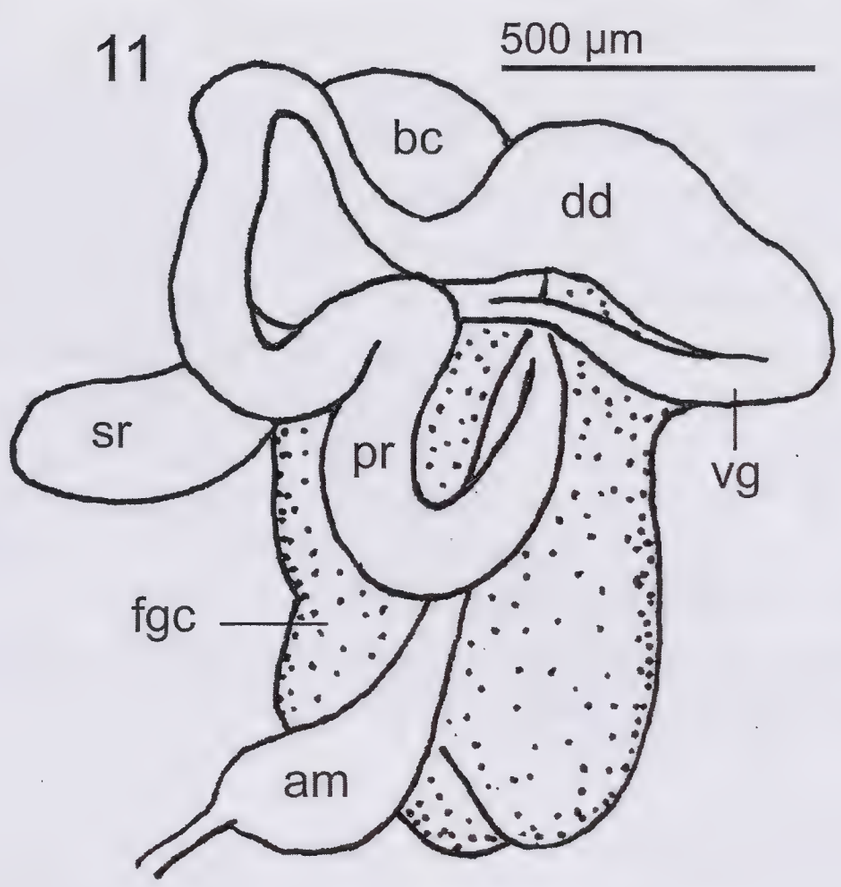
The phylogenetic relationships between *A. theveneti* new species, *A. trentinae* new species and other bluish-grey species could not be resolved with the data available, due to the small sample size and limited genetic data. Only sequence data of *A. albatrossae* is available for comparison. However, the COI sequences of *A. theveneti* new species and *A. trentinae* new species had a pairwise identity of 95.4%, which is similar to the pairwise identify between *A. albatrossae* and *A. theveneti* new species (96.4%) and between *A. albatrossae* and *A. trentinae* new species (95.6%). Considering that *A. albatrossae* is clearly morphologically distinct from *A. theveneti* new species and *A. trentinae* new species, this further supports the distinction of the two new species. Additional analyses including other bluish-grey species (*A. williamsi*, *A. erwinkoehleri*, and *A. andersoni*) will be necessary to further resolve the phylogeny of this group and possibly identify additional species. This is beyond the scope of this paper.

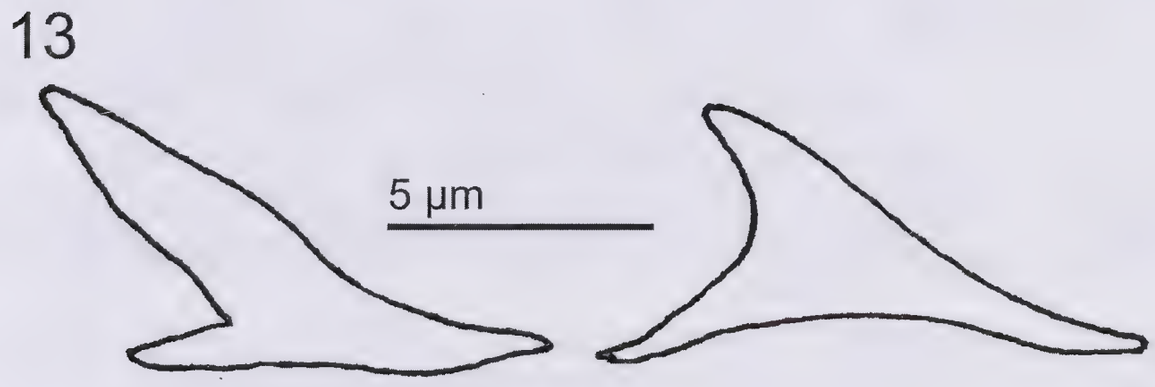


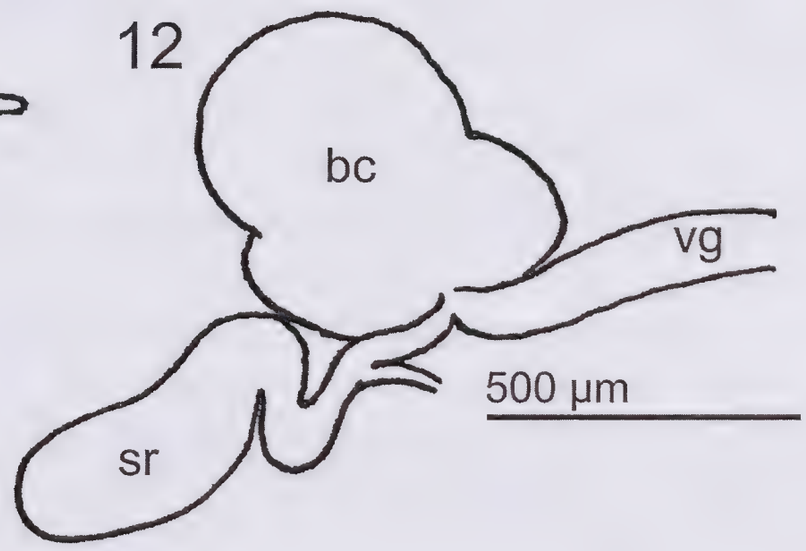


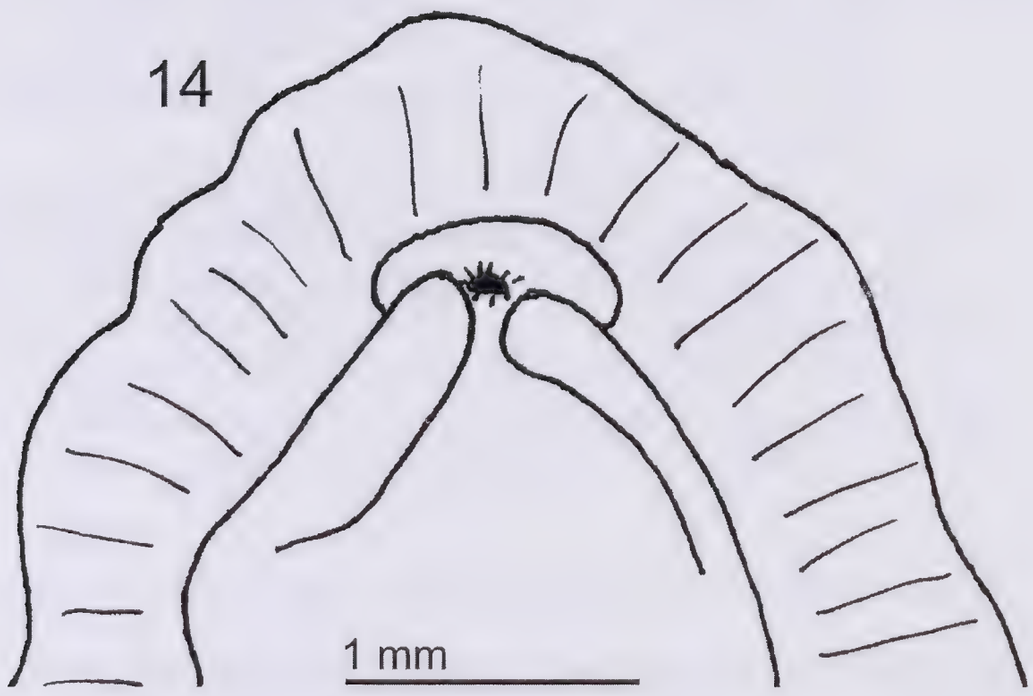
Figures 6–9. Scanning electron micrographs of the radula of specimens of ***Aldisa theveneti* new species** and ***A. trentinae* new species**. **6–7.** Specimen of ***A. theveneti* new species** from Mont-Dore, New Caledonia (MNHN IM-2013-86183). **6.** Detail of the radular teeth. **7.** Entire radula. **8–9.** Holotype of ***A. trentinae* new species** from St. Leu, Reunion (MNHN IM-2013-86182). **8.** Detail of the radular teeth. **9.** Entire radula.

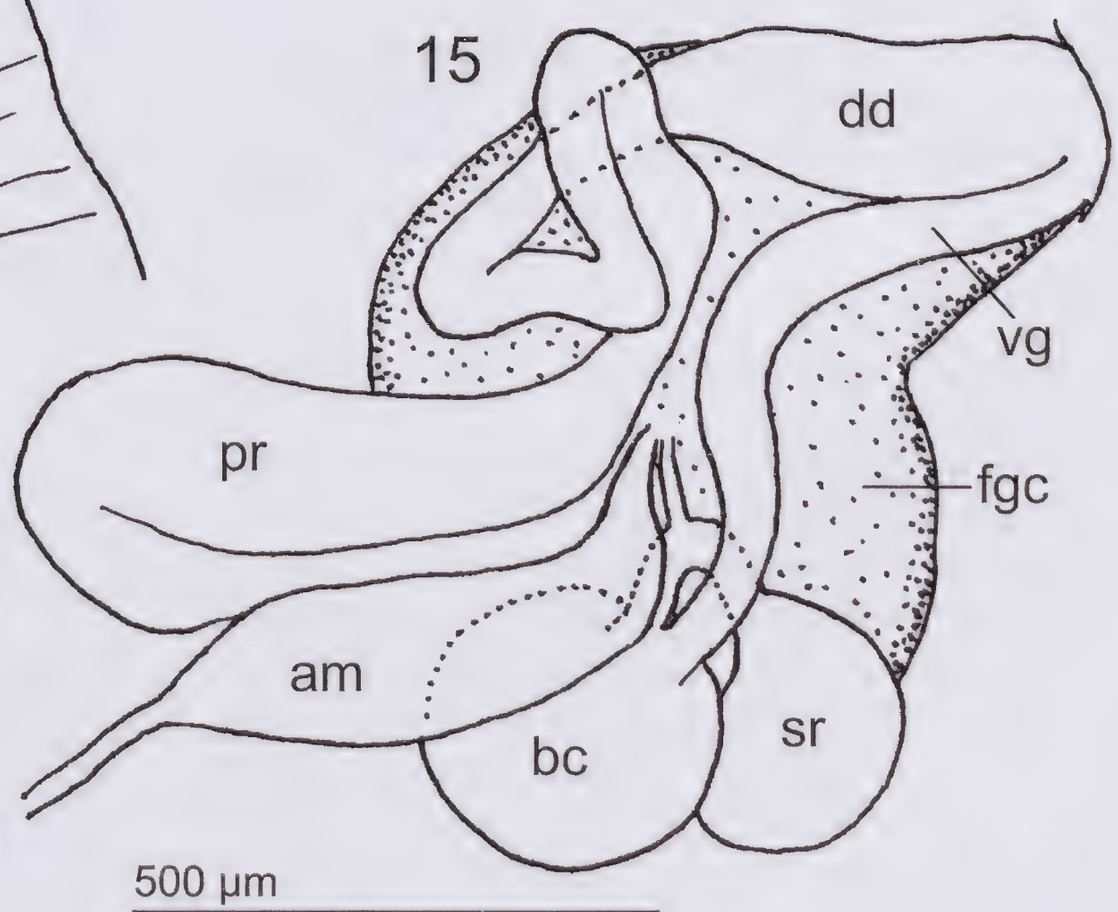


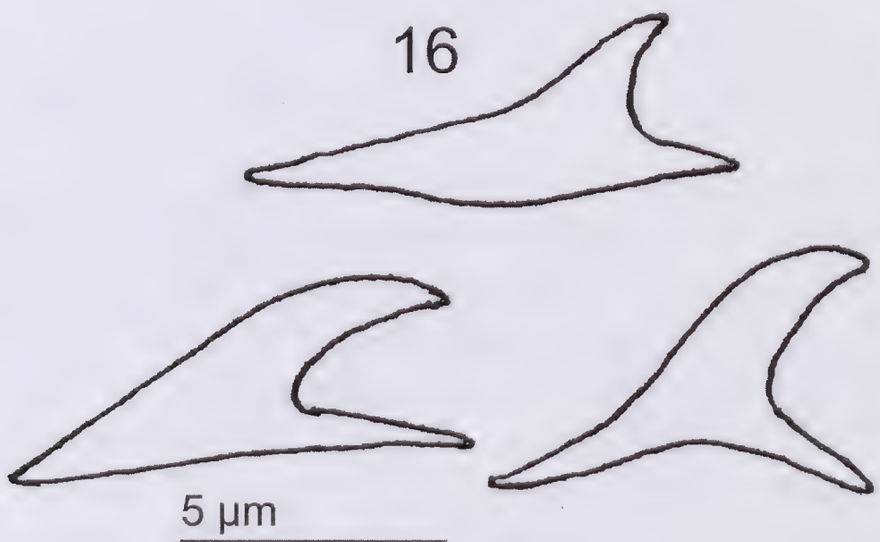












Figures 10–16. Drawings of the anatomy of specimens of ***Aldisa theveneti* new species** and ***A. trentinae* new species**. **10–13.** Specimen of ***A. theveneti* new species** from Mont-Dore, New Caledonia (MNHN IM-2013-86183). **10.** Anterior end of the foot. **11.** General view of the reproductive system. **12.** Bursa copulatrix and seminar receptacle connections. **13.** Penial spines. **14–16.** Holotype of ***A. trentinae* new species** from St. Leu, Reunion (MNHN IM-2013-86182). **14.** Anterior end of the foot. **15.** General view of the reproductive system. **16.** Penial spines. Abbreviations: **am**, ampulla; **bc**, bursa copulatrix; **dd**, deferent duct; **fgc**, female gland complex; **pr**, prostate; **sr**, seminal receptacle; **vg**, vagina.

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Bathyptychia shennong (Gastropoda: Stylommatophora: Clausiliidae), the first new species of terrestrial mollusk described from Shennongjia National Park, Hubei Province, China

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ABSTRACT

Bathyptychia shennong Chen and Liu, new species, is described and illustrated. It represents the first new molluscan taxon named from Shennongjia National Park, Hubei Province, China, a biodiversity hotspot. The new species is closely related to the type species, *Bathyptychia aplostoma* (Heude, 1885), but can be distinguished by its strong ribbing, a superior lamella that is weaker and not as pronounced, and deeper and smaller plicae.

Additional Keywords: taxonomy, land snail, Phaedusinae

INTRODUCTION

Bathyptychia Lindholm, 1925 is a clausiliid genus endemic to China. In previous studies, 19 species and subspecies had been assigned to three subgenera, *Bathyptychia* (s. str.), *B.* (*Strictiphaedusa*) H. Nordsieck, 2001 and *B.* (*Brachyptychia*) H. Nordsieck, 2001, were reported from Hubei, Jiangxi, Shaanxi, Sichuan, Chongqing, and Yunnan Provinces (Nordsieck, 2001, 2003, 2012, 2016; Hunyadi and Szekeres, 2016; Grego and Szekeres, 2020). *Bathyptychia* can be readily separated from others in the subfamily Phaedusinae by the small, slender, spindle-shaped shell with straight ascending and inferior lamella that ends deep in the shell. Its superior lamella is mostly continuous with the spiral lamella, and deep plicae are present: 3–9 short palatal plicae, more or less fused to the lunella in the part adjoining the lowest palatal plica (Nordsieck, 2001, 2012).

Shennongjia National Park, known as the roof ridge of central China, is located in the northwestern part of Hubei province and is a biodiversity hotspot (Xie et al., 2017). However, there are few records of terrestrial mollusks in numerous previous biological surveys, and no new molluscan taxon had been named from this region (Chen, 1992; Zhou et al. 2017). In this paper, a new species of *Bathyptychia* (s. str.) from Shennongjia National Park is reported.

MATERIALS AND METHODS

The living specimens were drowned in water before the shell and the soft parts were separated. The soft parts intended for anatomical study were preserved in 75% ethanol. To observe the inner structures through the thick shell, a glycerol solution or anhydrous ethanol was used to infiltrate the shell surface. Shells were measured using a vernier caliper. The nomenclature for the internal structures is based on Nordsieck (2007). Anatomical observations were done under an AmScope® SF-2TRA stereomicroscope. Photos of the shell were taken using a Canon® 5D Mark IV with Laowa® 25mm 2.5–5X Ultra Macro lens. Photos were stacked with Zerene Stacker® and modified in Adobe Photoshop® CC 2018. Photos of two syntypes of *Bathyptychia aplostoma* (Heude, 1885) (MCZ Malacology 167099 and MCZ Malacology 318086) were obtained from the official website of the Museum of Comparative Zoology, Harvard University (https://mcz.harvard.edu/) for morphological comparison.

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Institutional acronyms used in the paper are: BNZM: Broad Nature Zoological Museum (Wuhan, Hubei); CZY: private collection of Zhe-Yu Chen (Wuhan, Hubei); HBUMM: mollusc collection of the Museum of Hebei University (Baoding, Hebei); LZP: private collection of Zheng-Ping Liu (Chengdu, Sichuan); MCZ: Museum of Comparative Zoology, Harvard University (Cambridge, USA); SZ: private collection of Miklós Szekeres (Budapest, Hungary)

SYSTEMATICS

Family Clausiliidae Gray, 1855
Subfamily Phaedusinae A. J. Wagner, 1922
Genus *Bathyptychia* Lindholm, 1925

Type Species: *Clausiliia aplostoma* Heude, 1885, by original designation.

***Bathyptychia shennong* Z.-Y. Chen and Z.-P. Liu, new species** 神农深褶螺
(Figures 1–4, 6–7)

Diagnosis: A slender, small species with ribbed shell surface, cylindrical early whorls, detached peristome, superior and spiral lamellae connected, retracted inferior lamella, subcolumellar lamella not visible through the aperture, very long principal plica and ventral lunella including one moderately long upper palatal plica and one or two parallel or obliquely situated ovate plicae.

Description: Shell (Figure 1) small, consisting of 13½ to 14¾ slender and reddish-brown whorls, apical part attenuated. Protoconch of three narrow whorls, slightly wider than uppermost teleoconch whorls. Teleoconch with densely packed, regular axial ribs that gradually strengthen on lower whorls, ca. 12 ribs per mm on last whorl. Ribs weaken on umbilical area. Weak basal crest present near

Figure 1. ***Bathyptychia shennong* new species, holotype,** HBUMM 10028. Scale bar = 5 mm.

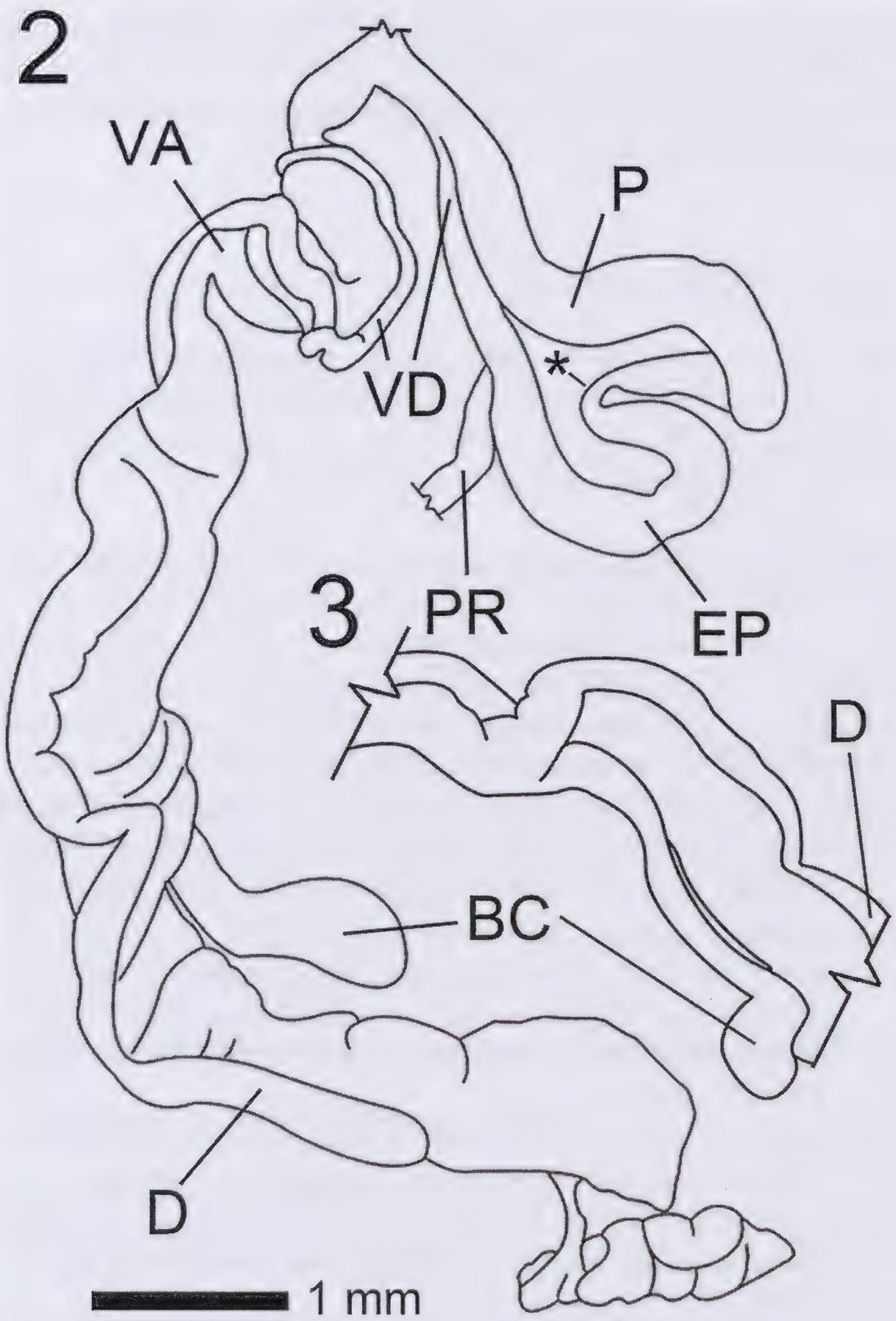

Figure 2–3. Genitalia of ***Bathyptychia shennong* new species**. Abbreviations: B, bursa copulatrix; D, diverticulum; EP, epiphallus; P, penis; PR, penial retractor muscle; VA, vagina; VD, vas deferens. Asterisk indicates the narrow transition between penis and epiphallus.

peristome. Aperture oval-piriform. Peristome expanded and detached from penultimate whorl. Lower left rim of sinulus internally concave. Superior lamella strong, connected to spiral lamella. Spiral lamella stronger at ventral side, inner end reaching penultimate whorl. Inferior lamella hidden, not visible through aperture, posterior part slightly shallower than spiral lamella (two specimens examined). Subcolumellar lamella not visible through aperture, deeply situated in shell. Principal plica starting at end of penultimate whorl and extending to behind peristome. Lunella ventral, except for relatively long upper palatal plica, one or two parallel or obliquely situated ovate plicae present (smaller plicae or "indentations" occasionally appearing on either side). Ovate plicae fuse into vertical lunella in few specimens. Clausilium plate length-width ratio about 3:2, not notched, invisible through aperture, outer surface of clausilium plate bears distinct spiny protrusion at rim of palatal side.

Genitalia (Figs 2–3). Penis moderately long, somewhat thick, simple outside. Epiphallus about half length and

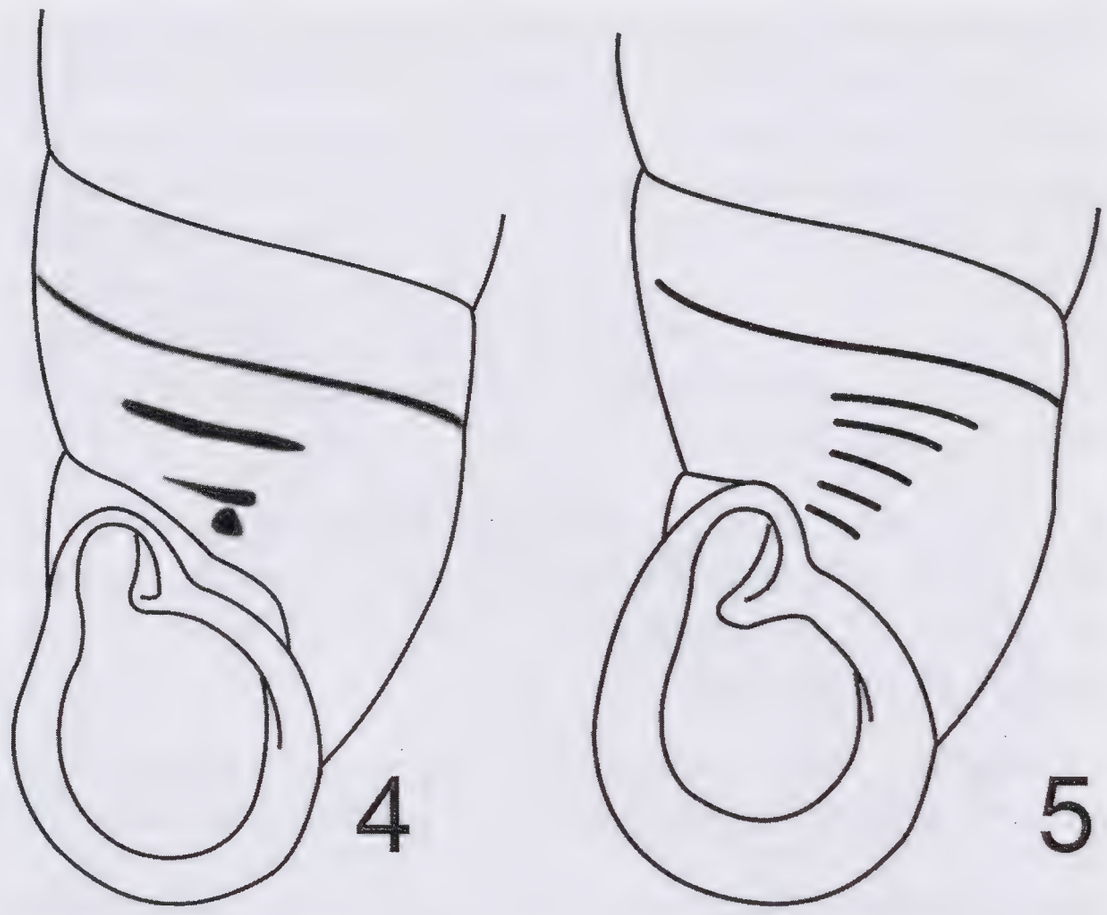

Figure 4–5. Plicae comparison of ***Bathyptychia shennong* new species** (4, holotype) and *Bathyptychia aplostoma* (Heude, 1885) (5, syntype of *Clausilia aplostoma*, MCZ Malacology 167099).

Figure 6–7. Satellite image of (6) and habitat at (7) type locality of ***Bathyptychia shennong* new species**.

same thickness as penis; joint between them thin. Penial retractor muscle situated at end of epiphallus. Diverticulum long, adherent to spermoviduct. Bursa copulatrix duct thin and about 2/3 length of diverticulum. Bursa copulatrix ellipsoid.

Measurements: Shell height = 13.5–14.8 mm, shell width = 2.6–3.0 mm (n = 9).

Type Material: Holotype, HBUMM 10028 [shell height = 14.1mm, shell width = 2.8mm]: 19 October 2019, leg. Zhe-Yu Chen, from type locality; Paratypes: 5 ethanol preserved specimens (HBUMM 10029 CZY/1), 7 empty shell (HBUMM 10030), 2 empty shells (HBUMM 10031, broken for internal structure observation), from type locality, 19 October 2019, leg. Zhe-Yu Chen; one empty shell (BNZM), from type locality, 18 April 2019, leg. Xiao-Ran Zhu.

Type Locality: Banbiyan [板壁岩], Shennongjia National Park [神农架国家公园], Shennongjia Forestry District [神农架林区], Hubei Province, China, 31.459239 N,110.222534 E, alt. 2630m.

Etymology: The species name comes from the ancient Chinese god, Shennong; treated as a noun in apposition.

Comparative Remarks: The new species resembles the type species *Bathyptychia aplostoma* in having deep and smaller plicae and a slender and fragile apical section, characters that distinguish both from other members of the genus. When compared with *B. aplostoma*, however, the new species is more strongly ribbed and its superior lamella is weaker and not as proounced, with deeper and fewer plicae (Figures 4–5). Moreover, the lower plicae of *Bathyptychia shennong* new species are usually shorter and mostly form rounded to triangular nodules (Figures 4–5). In addition, the slightly swollen protoconch seen in the new species does not occur on *B. aplostoma*.

Distribution: This species is known from the type locality only (Figs 6–7).

Ecology: All specimens were collected from humus under mosses covering high-altitude rock outcrops. The species probably feeds on fresh or rotted moss.

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Revision of two type selections in the Unionidae (Bivalvia) by Johnson and Baker (1973)

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ABSTRACT

In their catalog of the freshwater mussel types (Bivalvia: Unionoidea) at the Academy of Natural Sciences in Philadelphia, Johnson and Baker (1973) made a number of type selections that were not valid. Two of them are here reviewed and valid selections made based on specimens from both sets of material.

Text Acronym: ANSP, Academy of Natural Sciences of Drexel University, Philadelphia PA, USA.

Tritogonia nobilis (Conrad, 1854)

Unio nobilis Conrad, 1854: 297, figs. 2, 3.

LECTOTYPE SELECTION

Lectotype ANSP 43019, here selected (Figure 1)

Conrad figured two specimens but did not select a type. Johnson and Baker (1973: 163) stated:

nobilis, Unio: 1854, JANSP (2) 2: 297, pl. 27, figs. 2–3 (Bayou Teche, Louisiana). Lectotype, here selected, ANSP 43019a, figure 3.

ANSP 43019 is a lot of three specimens (originally four, according to the ANSP ledger record) donated by Emanuel R. Showalter, who obtained them from Conrad. The example selected by Johnson and Baker as the lectotype matches Conrad's figure 3 (Figure 2) in shape but not in sculpture, which Conrad's illustrator delineated very clearly. Johnson and Baker are thus considered here to have indicated two specimens, invalidating their selection.

Interestingly, one of the two other specimens in ANSP 43019 has a label in Conrad's hand pasted inside it reading "No. 3. to be returned". It is a smaller, male specimen of very different shape to his figure, and it is thus possible that the illustrator missed Conrad's instructions. Despite its only being recorded from "Louisiana" as opposed to Conrad's more specific locality in that state, "Bayou Teche", the original syntype status of this lot is not disputed, whereas the model for Conrad's figure is uncertain (see below). To preserve stability, therefore, the specimen indicated by Johnson and Baker is here selected as lectotype, retaining the number ANSP 43019. The two resulting paralectotypes are newly cataloged as ANSP 484508.

A second lot, ANSP 43038, contains four specimens (originally five) that donor "D. S. Sheldon" apparently obtained from Conrad. Although the Academy's display board is marked "Davenport, Iowa" this is probably not the locality. Professor David Sylvester Sheldon (1809–1886) of Iowa College in Davenport collected mollusks and donated specimens widely (Parry, 1893); the smaller label in this lot, presumed to be his, states "Lousiana", with "T. A. C" added in pencil. Under ICZN Article 72.4.1.1 (". . . any evidence, published or unpublished, may be taken into account to determine what specimens constitute the type series") this lot could thus also be original syntypes.

A reviewer has pointed out that several other figures in Conrad's paper seem not to match perfectly the shells since identified as the types. Whether this represents idealization on the illustrator's part (Daston and Galison, 2007) or is evidence that the specimen figured was genuinely different from the one since treated as the figured type cannot be objectively established unless a shell matching the figure exactly comes to light. In at least one other case, however (*Unio porrectus*: 296, pl. 26, fig. 7), the erosion pattern clearly depicted in the figure differs greatly from that on the specimen subsequently considered the figured type.

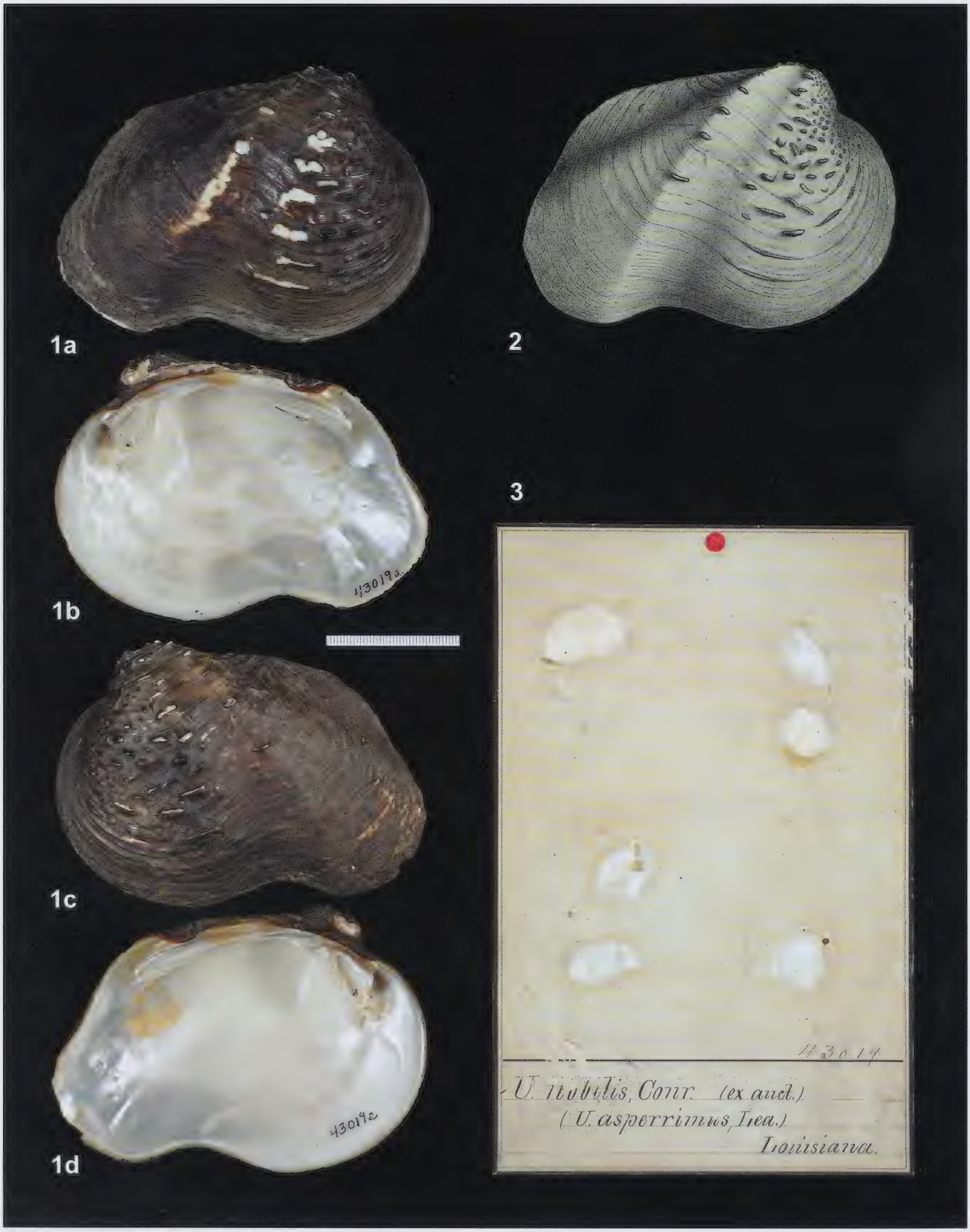
Quadrula quadrula (Rafinesque, 1820)

Obliquaria quadrula Rafinesque, 1820: 307, species no. 35.

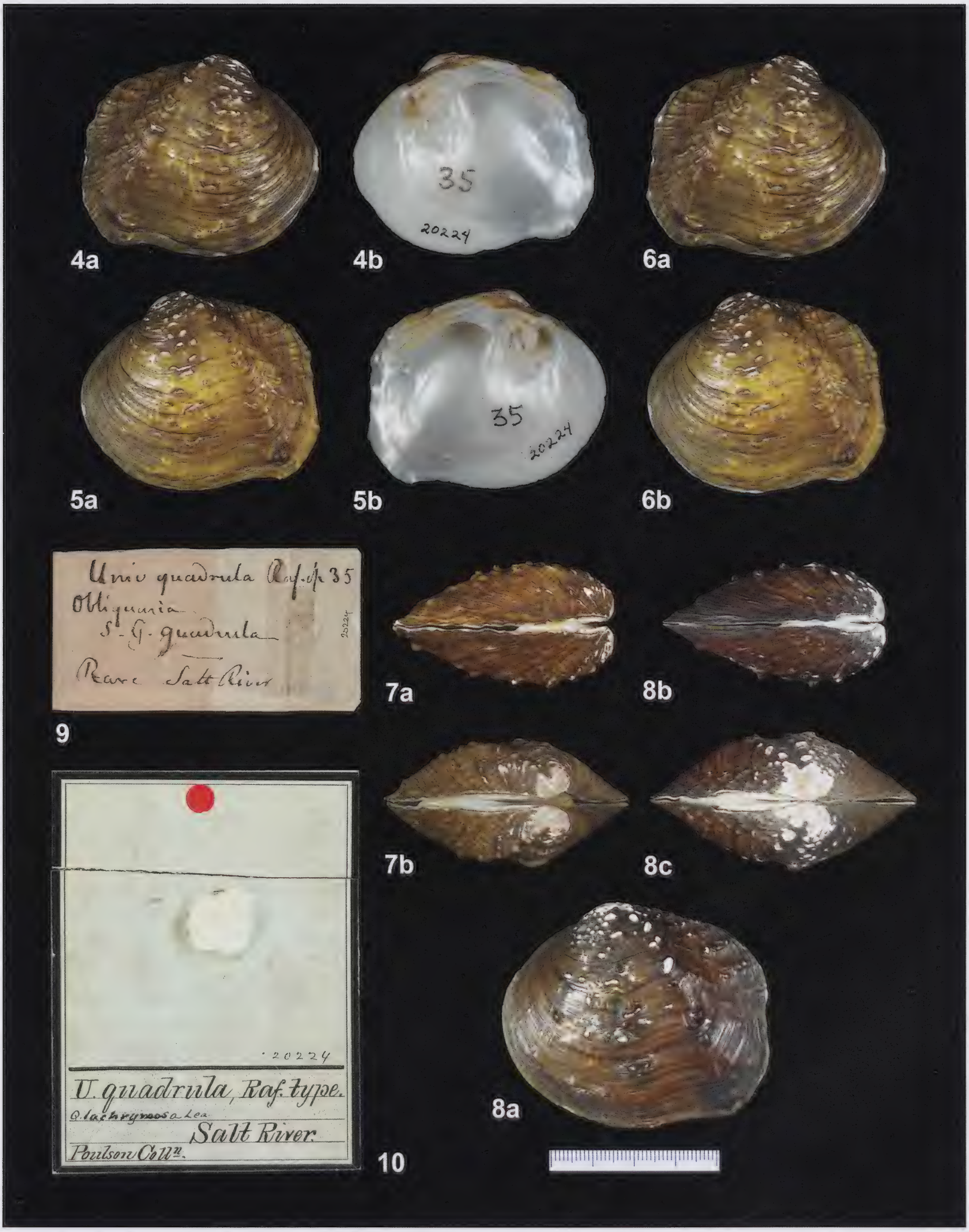
LECTOTYPE SELECTION

Lectotype ANSP 20224, here selected (Figs. 4, 6, 7)

Rafinesque gave a size range ("Largeur 2 à 3 pouces" = 54.1 to 81.2 mm) and proportions of length, height and width, but did not figure or measure a specimen. Poulson (1832: v) stated that "most of the shells described in the following pages have been placed in my cabinet by Mr. Rafinesque, with his labels and references". ANSP 20224



Figures 1–3. *Tritogonia nobilis* (Conrad, 1854). **1.** *Unio nobilis* Conrad, 1854. Lectotype, ANSP 43019, 113.4 mm maximum chord. **2.** *Unio nobilis* Conrad, 1854: figure 3, here adjusted to similar size. **3.** *Unio nobilis* Conrad, 1854, original ANSP display board, ANSP 43019.



Figures 4–10. *Quadrula quadrula* (Rafinesque, 1820). **4.** *Obovaria quadrula* Rafinesque, 1820. Lectotype, ANSP 20224, 59.7 mm maximum chord. **5.** *Obovaria quadrula* Rafinesque, 1820. Valve associated with lectotype in ANSP collection. **6.** *Obovaria quadrula* Rafinesque, 1820. Lectotype and associated valve conjoined in two views, showing overlaps. **7.** *Obovaria quadrula* Rafinesque, 1820. Umbonal and lateral views of lectotype and associated valve showing incomplete closure. **8.** ANSP 313992, *Quadrula quadrula* (Rafinesque, 1820) from the Ohio River in Hamilton County, Ohio, shown for comparison of closure. 64.9 mm maximum chord. **9.** Rafinesque's original label with ANSP 43019. **10.** Vanatta's display board for ANSP 43019. Rafinesque's label is glued to its back. Scale bar units: mm.

came from the Poulson collection and was cataloged in 1915. Malacology collection manager Edward G. Vanatta repeated at the time Poulson's assertion and added that "These shells are now in the collection of the Academy of Natural Sciences of Philadelphia" (1915: 549). Curiously, he cited 20224 as "this shell" and not "the type" as he did with almost all the others in his list, despite having entered them all the Academy's ledger under the heading "Rafinesque's types from the Poulson Collection" (original emphasis). Nevertheless, its inclusion in his list infers equal standing with the others as an original Rafinesque Collection lot and its shells are thus presumed syntypes. The label (Figure 9) affixed to the back of Vanatta's own display board (Figure 10) is in Rafinesque's hand, judging by comparison with his 1818 field book preserved and placed online by the Smithsonian Institution[1].

Rafinesque stated "quite common in the Ohio" ("assez commune dans l'Ohio") and this lot bears the locality "Salt River". This may be the "Salt Creek" recorded by Rafinesque in the above-mentioned field book on October 25, 1818, north of Zanesville, Ohio or perhaps the Salt River that joins the Ohio below Louisville, Kentucky.

It is not clear why Johnson and Baker (1973: 167, pl. 2, fig. 2) wanted to select this specimen to be the neotype rather than the lectotype. Their selection can be set aside, however, as it did not satisfy at least two of the requirements of Article 75c of the International Code of Zoological Nomenclature then in force (Stoll et al., 1964). Specifically, the authors did not provide "a statement of the characters . . . differentiating the taxon for which the neotype is designated", nor "the author's reasons for believing all the original type material to have been lost or destroyed, and the steps that have been taken to trace it."

This species is nowadays placed in the genus *Quadrula* Rafinesque, 1820, of which it is the type.

Examination of ANSP 20224 suggests that the two valves might not represent the same specimen. Their margins do not match exactly (Figure 6) and they are prevented from closing completely by contact between the lateral tooth and its receiving groove (Figure 7). In addition, their growth increments, though matching in position, are of differing strength, suggesting that they might be from the same locality and cohort but not the same specimen. Furthermore, the number 35 (from Rafinesque's list) was not inscribed simultaneously on both valves, as evinced by the different ink and the execution of the "3". The number on the valve in Figure 4 perfectly matches Vanatta's entry in the ledger, however.

Comparison with numerous specimens of *Q. quadrula* from the Ohio River in the ANSP (e.g., Figure 8) reveals some natural variability in closure and overlap and a definitive judgement is thus not possible. In view of this, and to preserve stability, the right valve (Figure 4) of ANSP 20224 is here selected as the lectotype of *Obliquaria quadrula* Rafinesque, 1820. Should some way be found to demonstrate unequivocally that the other valve belongs to the same specimen, then that would simply be incorporated into the lectotype.

[1]https://collections.si.edu/search/results.htm?q=record_ID%3Afbr_item_MODSI44

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